INFLUENCE OF EPICUTICULAR WAX ON HEAT AND DROUGHT TOLERANCE IN TAM 112 X TAM 111 POPULATIONS

A Dissertation

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ABSTRACT

High temperature and drought are the major constraints to wheat production globally. However, plants cope with stress by manipulating their physiological processes. This study investigates the role of leaf and glume wax content in reducing canopy temperature and quality stability in TAM 111 x TAM 112. A population of 124 recombinant inbred lines (RILs), derived from TAM 111 and TAM 112 was grown in greenhouse and multiple field locations across Texas for two years. The RIL showed variation for leaf wax and other physio-morphological traits indicating there is transgressive segregation for the traits. In this study, leaf wax didn't show any association with neither conductance nor fluorescence. In our field study, epicuticular wax showed positive as well as negative association with yield and yield components suggesting need for further research. In addition, QTL were detected in chromosomes 1B, 2B, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 6A, 6B, 6D, 7A, 7B and 7D. Among the 98 QTL, 18 loci across 10 chromosomes were associated with leaf epicuticular wax. For glume wax 14 QTL were identified with QTL explaining 7.6 to 13.6% variation. Yield and yield components QTL were found across the chromosomes. In addition, the RILs were analyzed for quality traits across locations. The protein content was negatively associated with yield parameters and mixograph peak time. Genotypic data was analyzed for co-localization between quantitative trait loci (QTL) regulating quality traits. QTLs were detected in all A, B and D genome. However, chromosome region of

7D followed by 1D had the most of the QTLs for traits including protein, peak time, hardness, kernel diameter, kernel weight, test weight, yield and wax. The QTL identified for some quality traits were consistent and stable across environments and could be useful in development of cultivars.

DEDICATION

To my beloved family for their unconditional love and support

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NOMENCLATURE

LF	Leaf wax
GW	Glume wax
LT	Leaf temperature
ST	Spike temperature
CF	Chlorophyll fluorescence
SC	Stomatal conductance
FLL	Flag leaf length
SL	Spike length
PL	Peduncle length
KNS	Kernel number per spike
SHW	Single head weight
Spm	Spike per meter squared
KW	Kernel weight
Yield	Grain yield/plot
QTL	Quantitative trait loci
TW	Test weight
HD	Kernel hardness
DIA	Kernel diameter
FL	Flour weight
PT	Mixograph peak time

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1. INTRODUCTION AND LITERATURE REVIEW

Wheat is an important, widely grown, versatile cereal crop in terms of harvested area and adapted to diverse environments (Mladenov et al. 2012; Gourdji et al. 2013). It is the staple crop in many countries and a main source of carbohydrates for both humans and livestock. Wheat production has increased since 1960s due to several factors, mainly, development of high yielding, semi-dwarf wheat varieties along with resistance to biotic and abiotic stresses and better agronomic practices (Semenov et al. 2012). It was reported that the genetic gain in wheat since then was about 1% per annum (Trethowan et al. 2002; Graybosch et al. 2010). Despite the projected need to increase wheat production from 720 million tons to 950 million tons by 2020 to feed the growing population, the top wheat producing nations are showing a decline in their yield improvement rate (Punia et al. 2011; Ray et al. 2013). The main factor for yield decline is due to increasing average growing temperature and persistent dryness in many wheat-growing regions of the world. Though wheat production is feasible in warmer areas, heat stress during anthesis and grain filling stage is a major constraint (Reynolds et al. 1994). High temperatures, even for a brief period during grain filling, result in drastic yield reductions (Hawker et al. 1993). Heat stress induces early senescence and accelerates grain filling and reduces carbon assimilation. Further, heat stress is confounded with moisture stress and contributes to severe yield loss.

However, plants have certain adaptations to cope with heat and drought stress. The adaptations, either physiological or morphological may differ in their response to such stresses depending on the stages of plant development, stress intensity and duration (Rahman et al. 2009). Though there are many traits associated with tolerance, they are not exploited in breeding programs due to the lack of suitable rapid phenotyping methods. Hence, rapid, repeatable across different genetic background and easily measurable traits are necessary to better understand the mechanism of tolerance and its association with yield components, to aid in breeding heat and drought tolerant genotypes.

1.1 Effects of heat and drought stresses on wheat growth

Wheat is sensitive to high temperatures (Wollenweber et al. 2003) and elevated temperature reduces the duration of all developmental stages and consequently impacts wheat production. Heat stress accelerates maturity, affects metabolic pathways, changes cell membrane structure, chlorophyll content and eventually plant senescence and yield loss (Dhyani et al. 2013). High temperatures during the double ridge stage affect spikelet initiation. Heat stress during anthesis can cause flower abortion, increased photorespiration and reduced carbon dioxide assimilation. In addition, it affects meiosis by inhibiting cell division, pollen growth and in turn the grain fertility (Al-khatib and Paulsen 1984; (Wardlaw et al. 1995). The grain set was found to be sensitive to high temperatures in the first three days after anthesis. The reduction in

grain-set was due to abnormal ovary developments, poor pollen dehiscence and pollen tube formation (Saini et al. 1982; Saini et al. 1983). Further, heat stress during grain development is also deleterious due to its effect on kernel number and kernel weight. The kernel number and weight along with number of spikes are the main components of total grain yield and a reduction in either kernel weight or number may lead to an overall reduction in yield. Temperatures of 35°C for ten days resulted in 29% and 36% reduction in both kernel weight and numbers respectively (Assad et al. 2002). Severe temperature stress during grain filling may cause early physiological maturity, shortening of grain filling duration, and in both yield and grain size reduction. In addition, it was found that a short period of early high heat temperature reduced grain growth to a greater extent than much longer periods of moderately high temperatures (Stone et al. 1995).

Further, a 10% negative yield response was observed for every 1°C increase in night-time temperatures above 20°C (Lobell et al. 2005). Elevated temperatures alter the source and sink processes as well as limits translocation of photosynthates to the kernels and thereby affects grain weight (Jenner 1994). Further, it damages the physiological processes via denaturation of enzymes, formation of reactive oxygen species (ROS) and changes in membrane integrity, thylakoid structure, mitochondrial activity and lipid metabolism (Maheswari et al. 1999). Photosynthesis is highly sensitive to heat stress and high temperatures greatly reduce the activities of photosystem II (PSII) and accelerate photorespiration (Camejo et al. 2005). It also

impairs chlorophyll biosynthesis along with changes in senescence related metabolic activities due to the electrolyte leakage of thylakoid membrane (Ristic et al. 2007). Further, increase in proteolytic activity was observed as senescence progressed due to high temperature (Al-khatib and Paulsen 1984).

Apart from high temperatures, drought stress is an additional constraint to wheat production. Wheat is grown as a rain-fed crop in many areas, specifically in semiarid regions. Due to the variation in rainfall, low soil moisture along with high heat limits wheat production. High temperatures and drought stress, collectively or individually are the major constraints to wheat yield, globally (Pradhan et al. 2012). It has been reported that drought along with heat is the major limitation to maximum wheat production in the Great Plains of the United States (John 1983). The moisture stress that occurred in Southern Great Plains in 2011 resulted in a loss of $243 million in wheat production alone (Xue et al. 2014). Like heat stress, drought stress decreases accumulation of stem reserves. It was found that up to a 23% decrease in the main stem weight occurred when wheat was subjected to drought stress (Ehdaie et al. 2006). Drought stress during early booting to anthesis is known to inhibit all growth and development stages including germination, delay in root growth, stomatal closure and wilting. It also reduces tiller production, spikelet formation and kernel weight and eventually, crop productivity (John 1983). In addition, water stress induces male sterility and failure of pollen development by abnormal vacuolization of tapetal cells, disorientation of reproductive cells, desiccation of sporogenous tissue and down

regulating transcription of vacuolar (Ivr5) and cell-wall (Ivr1) encoding genes (Saini et al. 1982; Lalonde et al. 1997; Koonjul et al. 2005). Drought stress causes severe damage to photosynthetic process by affecting chlorophyll, PSII and Rubisco. Drought impairs the activity of ribulose 1, 5-bisphosphate carboxylase/oxygenase resulting in reduced photosynthesis (Bota et al. 2004). A decrease in photosynthesis could result from inhibition of PS II that in turn results in decrease in variable chlorophyll fluorescence. Further, drought stress decreases stomatal conductance leading to stomatal closure with increasing vapor pressure deficit (Maherali et al. 2003). In addition, drought stress elevates xylem pH due to increased ABA concentration that ultimately leads to stomatal closure (Wilkinson 1999). Though, drought is the major factor affecting water status of the plants, the severity is increased in the presence of heat.

The increasing incidence of heat and drought in relation to yield is a major focus of many wheat breeding programs worldwide, as it could undermine future global food security. Thus, re-evaluation of the adaptive traits that are strongly associated with high and stable yield under heat and drought conditions is the most important criteria to prevent yield losses in the future.

1.2 Physiological and morphological traits associated with improving heat and drought tolerance

Heat and drought stress has major impact on the physiology of the wheat crop. Plants usually undergo pre and post-stress physio-molecular changes to better adapt

with the stress effects. Some of the adaptations include leaf waxiness, trichome density, stay green, osmotic adjustment, changes in metabolites such as proline, malate and aminobutyrate as well as sugars and polyols (Reynolds et al. 1998). Leaf epicuticular wax (EW) is a lipid layer outside the leaf cuticle layer. It was found to decrease transpiration rate and increase water-use efficiency under drought conditions. Studies reported that non-waxy lines did not perform as good as that of waxy lines under drought (John 1983). Similar to EW, trichomes protect the plants by reducing absorption of solar radiation and thereby minimizing the heat load of the canopy (Huttunen et al. 2010). Stay-green or chlorophyll retention is an indicator of stress adaptive mechanism and has been associated with increased yield and protein concentration in the winter wheat population (Lopes et al. 2012).

Plants maintain a favorable water status for survival in dry environments by accumulating solutes in the plant cells. Osmotic adjustment (OA) is associated with the maintenance of high cell turgor potential and plants tolerate drought by maintaining sufficient cell turgor to allow metabolism to continue under stress conditions (Dacosta et al. 2006). Further, OA induced turgor maintains cell elongation and contributes to enhanced root growth and soil water extraction under drought. Transpiration under high temperature reduces leaf surface temperature and lead to transpirational cooling. Canopy Temperature Depression (CTD) is considered as a heat escape mechanism and is a robust indicator of overall plant water status and can be used as a selection criteria for improved tolerance to heat and drought (Karimizadeh et al. 2011). Canopy

temperature (CT) depends on the quantity of water transpired by the leaves and stomata conductance. It was found that the genotypes with higher CT at early stage will conserve moisture by reducing transpiration and use it for later stages, thus making the canopy cooler (Abdipur et al. 2013). Further, CTD had a high correlation with yield in both heat and drought environments (Reynolds et al. 1998). Also, several authors (Johnson et al. 1982; Clarke et al. 1988) have reported a significant association among leaf glaucousness, reduced leaf CT and grain yield. CT is highly correlated with stomatal conductance in different environments (Rebetzke et al. 2012) and it was also found that drought resistant cultivars had higher stomatal and cuticular resistance. Like CT, chlorophyll content is another physiological trait that can be used with ease even for large population. It provides an estimation of photosynthesis, as high chlorophyll content under stress indicates reduced rate of photo-inhibition of the photosynthetic machinery and is associated with grain yield. Chlorophyll fluorescence has been widely used as a non-destructive and rapid method to estimate quantum yield of photosystem II. The maximum quantum yield of PS II is calculated as the ratio of variable fluorescence (Fv, a difference between maximum and minimum fluorescence) to maximum fluorescence (Fm) (Rohacek K 2002).

Another important trait associated with heat tolerance is cell membrane stability. The membrane thermo-stability has been expressed in terms of electrolytic conductance and measured by the cellular membrane stability assay (CMS) and tetrazolium triphenylchloride (TTC) assay. A significant correlation has been observed

between the CMS and yield under high temperature stress in wheat (Blum et al. 2001). The TTC assays were found to be reliable assays for heat tolerance due to their association with membrane stability and high heritability (Ibrahim et al. 2001)(Post-Beittenmiller 1996). Other morphological traits associated with stress avoidance or tolerance are leaf rolling, accelerated leaf senescence, leaf shading, tiller death, reduction in leaf expansion, awns and increased reflectance through leaf pubescence.

1.3 Epicuticular wax as an adaptive mechanism to stress in wheat

Plants are exposed to an array of biotic and abiotic stresses and in response they have evolved a multitude of defense mechanisms to protect from adverse environments. Leaf wax is one such adaptation to drought and heat stress. The cytoplasmic membranes are sites of epicuticular wax synthesis and are composed of complex acyl lipids (Jenks and Ashworth 1998). The waxes are complex mixtures of very long-chain fatty acids, alkanes, aldehydes, primary and secondary alcohols, ketones, esters, triterpenes, sterols, and flavonoids. The biosynthesis of epicuticular waxes is a complicated and dynamically regulated process (Jenks and Ashworth 1998). The initial process in wax biosynthesis begins with the elongation of C16 to C18 fatty acid precursors. The acyl chains undergo basic reactions of condensation, reduction, dehydration, and a second reduction for each of the two carbon elongations (Post-Beittenmiller 1996). The elongation systems involved in wax biosynthesis may be both sequential (generating a homologous series) and parallel reactions (generating different

lipid classes). The parallel pathways leading to the production of different wax classes are (a) decarbonylation, (b) acyl-reduction, and (c) β-ketoacyl-elongation. All three pathways are found in most of the plants species, but their contributions to the cuticular wax composition vary within and between species (Jenks and Ashworth 1998). Among the pathways, β-ketoacyl-elongation pathway results in the production of β-diketones and their derivatives, which are the major components of the cuticular wax of wheat and barley spike, leaf sheath, and internode (Wettstein-Knowles et al. 1980; Wettstein-Knowles 1987). There are several groups of genes involved in the wax biosynthesis and transportation. It was found that mainly CER and GL genes of Arabidopsis and maize encode proteins that are involved in wax transport (Lemieux 2014).

Apart from the amount of wax, structure and composition of wax is also influenced by temperature, light and humidity. For example, high temperature favors plate like wax structure, whereas, low temperature influences the formation of rods and tube like structure. Also, the duration of photoperiod influences the chain length of waxes in tobacco suggesting role of phytochrome in wax production (Jenks and Ashworth 1998). Leaves reflect excess radiation and the amount of reflection is influenced by the wax layer. The reflection property of wax has been studied between waxy and non-waxy leaves of various species. In Eucalyptus, glaucous species showed reduction in reflection and increase in photosynthesis than non-glaucous species. Further, a reduced absorption of incidence radiation can lower canopy temperature,

thus reducing transpirational water loss (Shepherd and Wynne Griffiths 2006). In many plants, increase in wax deposition is a response to heat and water stress. Stress resistant plants have thicker wax than the susceptible plants (Cominelli et al. 2008). It was found that canopy temperature in glaucous durum wheat grown under drought condition was 0.7°C cooler than non-glaucous (Richards et al. 1986). Furthermore, when Medicago WXP1 (wax production gene) was overexpressed, it induced wax production and increased drought tolerance in transgenic alfalfa (Zhang et al. 2005). Apart from these stresses, exogenous ABA also induces wax deposition (Panikashvili et al. 2007). The epicuticular wax not only helps in reflecting excess radiation but also keep the canopy cooler (Shepherd et al. 2006). Thus in this study, the influence of EW in different heat and drought stressed environments was tested to determine the relation between wax and stress avoidance.

1.4 QTL mapping of morpho-physiological traits in wheat

Heat and drought tolerance brings about many changes in gene expression and it is necessary to identify potential candidate genes for various adaptive traits by deploying unique technology (Khan et al. 2010). Mapping QTLs of agronomic interest helps to dissect genetic loci regulating complex traits. These QTLs can be used in marker-assisted selection (MAS) to speed the breeding process (Campbell et al. 2003; Pinto et al. 2010).

However, yield, and heat and drought tolerance are complex traits, controlled

by multi-loci with high genotype and environment (GxE) interactions and the QTLs identified in one environment are often difficult to identify in another environment due to the high GxE interaction (Budak et al. 2013). Most of the QTLs identified thus far are yield and yield components QTL, as yield is the most critical trait. Two QTLs for grain filling duration under heat stress were detected on chromosome 1B and 5A explaining 23% of the total variation (Yang et al. 2002). QTLs for grain yield, CT, NDVI and chlorophyll content were identified in chromosomal regions of 1B, 2B, 3B, 4A and 5A in wheat grown under high heat, drought and control environment (Pinto et al. 2010). In a multi-environment QTL analysis, CT and leaf porosity QTLs were identified along with plant height QTL in chromosomes 4B and 4D (Rebetzke et al. 2012). In another study, QTLs associated with heat susceptibility index and flag leaf glaucousness were identified on chromosome 1A, 2A, 2B and 3B and 5A respectively (Mason et al. 2010). Yet another study identified QTL for glaucousness on chromosome 3A explaining 52% of phenotypic variations (Bennett et al. 2012). Another study from our lab has identified QTLs for EW on chromosome 5A and 1B (Mondal et al. 2015). As discussed here, relatively very few studies have identified QTLs for glaucousness and epicuticular wax. Thus, identification of QTL for EW and its association with other agronomic traits of interest could be a potential tool to screen for heat and drought tolerant lines.

1.5 Rationale and objectives

The primary objective of this study is to understand the role of epicuticular wax and its association with heat and drought tolerance adaptation. Based on the research at our lab and the fact that epicuticular wax can reduce cuticular conductance and leaf temperature by reflecting incoming solar radiation, we hypothesize that understanding the role of epicuticular wax in heat and drought tolerance may allow development of stable varieties and secure wheat yield in high temperature regions.

Objective 1: Investigate the influence of epicuticular wax quantity and its association with leaf and spike temperature, stomatal conductance, leaf fluorescence and grain yield in controlled and heat stress treatment under greenhouse conditions. The hypothesis is that the epicuticular wax will lower stomatal conductance and leaf surface temperature by reflecting excess solar radiation and thus improving the adaptation to heat tolerance. The study was conducted in cultivars TAM 112 and TAM 111 and a set of 124 recombinant inbred lines (RIL) derived from TAM 112 and TAM 111. Both TAM 112 and TAM 111 are hard red winter wheat varieties, developed by Texas A&M AgriLife Research. The two varieties are adapted to limited irrigation areas and perform well under drought conditions. Heat tolerance was determined by comparing the association between wax quantities, canopy temperature and yield components both in heat and controlled greenhouse conditions.

Objective 2: Identify phenotypic correlation between physiological and morphological traits at different field locations across TX. In addition, identify QTLs associated with epicuticular wax, canopy temperature, yield and yield components across environments. The hypothesis is that the stable and major QTLs regulating leaf wax and stress tolerance will improve yield stability. The TAM 112 x TAM 111 population was grown in various environments across Texas for two seasons, 2012 and 2013. The locations were: College Station, Uvalde, Chillicothe, Bushland and Etter, TX. The phenotypic and genotypic data collected for the RILs were used to identify the genetic loci linked with wax and yield traits in the populations.

Objective 3: Determine the association between quality traits, wax and yield of the RIL population grown at different environments. The hypothesis is that the cooler leaf and spike temperature and unaltered rate of photosynthesis of waxy plants may increase the distribution of assimilates thereby increasing grain yield and quality stability. The RIL population grown under various locations and treatments were analyzed for quality based on kernel texture and milling characteristics.

2. INFLUENCE OF HIGH TEMPERATURE STRESS ON PHYSIOLOGICAL AND PHENOLOGICAL TRAITS OF TAM 112 X TAM 111 RECOMBINANT INBRED LINES

2.1 Introduction

Wheat (*Triticum aestivum L.*) is an important cereal crop around the world and stands first in terms of acreage and third in world production (FAO, 2013). The optimum temperature for the growth and development of wheat is approximately 15-25°C, but a constant or temporary occurrence of high temperatures (>31°C), especially during reproductive and grain filling stage lead to severe yield loss in many wheat growing regions of the world (Ferris et al. 1998). Although the response of heat stress varies at different phenological stages, changes in each stage such as increase in respiration, inhibition of starch synthesis and reduction in photosynthesis lead to reduction in overall yield (Ayeneh A et al. 2002). Further, increase in greenhouse gas concentrations and variable precipitation patterns due to climate change also influences wheat production. In wheat, annual yield loss due to global warming is estimated at US $7.7 billion, but it could rise to US $18 billion, by 2025 (Kumar et al. 2013).

Though progress has been made over the years to increase the productivity of wheat grown in stress environments, most of the current varieties still show susceptibility to heat stress (Hays et al. 2007). This is due to the complexity of the

trait that is controlled by many different genes (Kumar et al. 2013) and non-availability of effective selection criteria and phenotyping methods to identify heat tolerant genotypes. This necessitates screening of phenotypic traits that are easy to measure and valid on different genetic backgrounds in evaluating heat tolerance lines.

Plants have evolved certain mechanisms to cope with heat and drought stress conditions. Among many adaptations, epicuticular wax (EW) is an important adaptive trait that represents the interface between the plant and its environment. EW gives the plant surface a shiny, glossy and whitish bloom appearance, known as "glaucous"(Post-Beittenmiller 1996). Glaucousness has been associated with improved yield in wheat and barley. EW acts as a barrier between the plant and its environment by limiting water loss and reflecting and reducing the solar radiation on the leaf surface. The role of EW in improved water use efficiency, surface reflectance and resistance to leaf and stem diseases have been well documented (Clarke and Richards 1988). Further, EW had a significant effect on decreasing canopy temperature in sorghum and pinus seedlings (Pinus sp) (Thames 1960). Canopy temperature (CT) is an indicator of plant metabolic and physiological response to elevated temperatures. Due to its ease of measurement, it is widely used as a screening tool for heat tolerant germplasm (Reynolds et al. 1994; Amani et al. 1996). Stomatal conductance (SC) is highly sensitive to small changes in canopy and air temperature, influences the leaf transpiration rates and can be used

as selection criteria for improved adaptations in different environments. The differences in SC lead to variation in CT (Rebetzke et al. 2012) and it was found that an increase in stomatal conductance increases canopy temperature depression and increased grain yield in irrigated wheat. The photosynthetic efficiency is studied through chlorophyll fluorescence (CF), which provides information about the status of photosystem II and was used to distinguish the effect of water stress from photo-inhibition in wheat genotypes (Sayed 2003). Improved drought tolerance and increase in yield components in durum wheat genotypes were associated with increased chlorophyll content (Karimizadeh et al. 2011).

In cereal crops, flag leaf is an important source of carbohydrate production, which contributes to grain fill and thereby, determines yield potential (Li et al. 2012). It is the greatest contributor to yield because it stays green for longer than rest of the leaves and its short distance to spike. The leaf architecture and area are important while breeding for stress environments. It was found that the short leaves had the potential to withstand drought (Villegas et al. 2007). Flag leaf area had positive correlation with yield and yield components (Chowdhry et al. 1976). Likewise, spike length is another important component of yield and its contribution to final grain weight varies between genotypes and environmental conditions. The spike length had positive association with grain yield and it was suggested that selection of longer spike might increase grain yield due to its longer duration as a stay green organ and its closer distance to the grains (Hsu et al. 1971; Okuyama et

al. 2005). However, the relations between EW and the above-mentioned physio-morphological trait have not been evaluated in detail. We hypothesize that EW may influence some of the traits by reducing heat load of the plant and contributing to yield increase. The objective of the study is to understand the:

(i) influence of leaf wax and its association with physiological traits such as leaf temperature, spike temperature, stomatal conductance and fluorescence

(ii) morphological traits such as flag leaf length, spike length and peduncle length and

(iii) yield components under short term high temperature stress.

2.2 Materials and methods

2.2.1 Plant materials

The study was conducted in a greenhouse with a set of 124 recombinant inbred lines (RILs) derived from a cross between hard red winter wheat TAM 112 and TAM 111 in the year 2012 and 2013. Both parents and RILs were germinated and vernalized in petri dishes at 4°C for six weeks. The seedlings were transplanted at four plants per pot (12 x 15 cm) filled with Metro Mix 900 mixture (Sun Gro Horticulture, Canada) and later thinned into two plants per pot. Plants were fertilized with Peters 20:20:20 and were arranged in completely randomized design and replicated twice in the year 2012 and three times in 2013 each for control and heat stress treatments. Plants were grown in the greenhouse under optimal

conditions at 21°C/18°C day/night cycles with a 12 hr photoperiod from 7 a.m. to 7 p.m. with 600 µmol m^{-2} s^{-1} PAR supplemental light. At seven days after pollination (DAP) half of the RILs were transferred to a high temperature greenhouse set at identical conditions except for temperature which was set at a 38°C/21°C day and night cycle. Plants were kept under high temperatures for three days. The plants at the heat temperature greenhouse were watered daily to ensure adequate soil moisture. After three days the plants were moved back to the controlled chamber and grown until maturity.

2.2.2 Wax quantification

Four leaf discs of 0.8 cm in diameter samples from flag leaf were collected for wax analysis at 10 DAP using paper punches. Leaf wax was extracted and quantified using colorimetric technique as described (Ebercon et al 1977) Leaf wax was extracted with 1ml HPLC grade chloroform for 30 seconds, transferred to a clear 1.8 ml glass gas chromatography vials (GC) (VWR Auto sampler Vial) and vacuum dried. The dried extract was oxidized with 300 µl acidified potassium dichromate ($K_2Cr_2O_7$) and heated for 30 minutes (min) at 100 °C in a water bath. Later, 700 µl of deionized water was added to the vials and allowed to cool for color development. Thereafter, 100 µl samples were loaded in 96 well clear polystyrene plates (Greiner Bio-One, USA) and were analyzed using spectrophotometer (PHERAstar plus, BMG LABTECH) to determine the optical

density at 590 nm. Following the same extraction procedure, a standard curve was plotted from randomly selected flag leaves and the resulting wax-chloroform was used for serial dilution technique. The standard curve was used to calculate wax levels based on leaf area.

2.2.3 Physiological measurements

Flag leaf and spike temperatures were taken using a handheld infrared thermometer (Fluke 566 series, Everett, Washington, USA). The thermometer was held at 45^{o} degree angle to the leaf and spike and the measurements were taken between 12 pm to 3 pm. Flag leaf stomatal conductance (SC) was measured using a handheld leaf porometer (Model Sc-1, Decagon Services Inc, Pullman, WA) while, chlorophyll fluorescence measurements were taken with a handheld fluorometer (Fluoropen FP100). All the readings were taken at 10 DAP and on the flag leaf. Spike temperature, fluorescence and stomatal conductance were measured only in the year 2013.

2.2.4 Morphological measurements

Flag leaf length was measured from the base of the leaf to the tip of the leaf and the spike length was measured from the base of the spike to the top of the spike excluding awns. Peduncle length, the last inter node of main stem were also measured. The lengths of leaf, spike and peduncle were recorded at physiological maturity.

2.2.5 Statistical analysis

All the data was analyzed using statistical software SAS (SAS v9.2). The procedure PROC MEANS was also used for descriptive statistics. Pearson's correlations PROC CORR was used for determining the association between traits and treatments.

2.3 Results

2.3.1 Wax quantification

Phenotypic data collected on different traits for the TAM 112 x TAM 111 RIL population and the parental varieties are presented below in Table 1. The minimum wax content for TAM 111 in 2012 and 2013 were 3.04 and 4.8 mg/dm^{-2} and the maximum were 5.75 and 5.5 mg/dm^{-2} in control treatment whereas, for heat treatment it varied from 4.05 and 5.17 mg/dm^{-2} to 6.15 and 6.76 mg/dm^{-2}. The minimum and maximum wax content for TAM 112 in year 2012 ranged from 2.96 – 4.23 mg/dm^{-2} and 2.53 – 6.57 mg/dm^{-2} whereas in 2013 it ranged from 5.0 – 6.3 mg/dm^{-2} and 4.2 – 7.17 mg/dm^{-2} respectively (Table 1). No significant differences in the wax levels were detected between the parents in both control and heat treatments. The wax content among the RILs ranged from 1.33 – 8.52 mg/dm^{-2} in control treatment and 2.12 – 9.26 mg/dm^{-2} in high temperature treatment in 2012. In 2013, the wax content for RIL ranged from 3.0 -9.2 mg/dm^{-2} and 2.8 – 10.5 mg/dm^{-2} in control and heat treatments respectively.

Table 1 Range of physio-morphological trait values for parental cultivars and RILs under control and high temperature greenhouse conditions in years 2012 and 2013

	Control			High temperature		
Traits/units	**TAM 111**	**TAM 112**	**RIL**	**TAM 111**	**TAM 112**	**RIL**
KW (g)	1.3 – 4.4	2.2 – 6.2	0.30 – 8.3	0.8 – 5.2	1.0 – 5.9	0.10 – 8.0
KNO (no)	6.0 – 37.0	8.0 – 38.0	5.00 – 72.0	6.0 – 26.0	11.0 – 34.0	2.0 – 56.0
LT (° C)-2012	22.3 – 26.6	20.4 -24.6	20.5 – 26.6	31.5 – 34.7	31.5 – 34.7	26.4 – 36.5
LT (° C)-2013	22.6 – 27.3	24.6 – 27.2	22.7– 32.1	34.1 – 39.4	29.9 – 36.0	23.3 – 40. 7
ST (° C)	25.8 – 27.9	24.2 – 27.2	23.0 – 31.6	33.4 – 36.6	33.7 – 36.6	25.5 – 38.8
SC (mmol m^{-2} s^{-1})	22.9 – 318.6	96.4 – 286.9	20.2 – 492.0	29.5 – 349.6	39.6 – 358.7	10.3 – 512.6
FL (Fv/Fm)	0.71 – 0.75	0.71 – 0.75	0.46 – 0.79	0.60 – 0.74	0.65 – 0.74	0.32 – 0.79
FLL (cm)	13.6 – 18.7	18.3 – 23.6	2.2 – 32.0	8.6 – 26.0	13.7 – 25.2	7.4 – 32.1
SL (cm)	7.8 – 11.6	6.6 – 10.1	4.6 – 14.4	6.7 – 9.6	6.6 – 11.6	3.4 – 12.7
PL (cm)	2.0 – 8.7	1.5 – 9.2	0.8 – 18.4	1.0 – 8.2	3.0 – 14.4	0.4 – 17.1
Wax (mg/dm^2)-2012	3.04 – 5.75	2.96 – 4.23	1.33 – 8.52	4.05 – 6.15	2.53 – 6.57	2.12 – 9.26
Wax (mg/dm^2)-2013	4.8 – 5.5	5.0 – 6.3	3.0 – 9.2	5.17 – 6.76	4.2 – 7.17	2.8 – 10.5

KW-Kernel weight, KNO-Kernel number, LT, ST-Leaf and spike temperature, SC-Stomatal conductance, FL-Fluorescence, FLL-Flag leaf length, SL-Spike length, PL-Peduncle length

Among the yield components, though the kernel weight for RIL was comparable between the treatments, kernel number showed variation between high temperature treatments (2 to 56) compared to control (5 to72) (Table 1). The RIL population had significant variation for canopy temperature. Leaf temperature was taken in both years 2012 and 2013, whereas the spike temperature was taken only in 2013. The leaf temperature in the RIL population ranged from 20.2°C to 32°C in control and 23.3°C to 40.7°C in high temperature treatment in both years combined. The spike temperature among the RILs ranged from 23°C to 31.6 °C in control and 25.5 to 38.8°C in heat treatment (Table 1). For stomatal conductance and fluorescence the RIL showed transgressive segregation as that of other traits. The RIL showed extreme values than the parents for all morphological traits including flag leaf length (FLL), spike length (SL) and peduncle length (PL) in both control and heat treatment. Due to some unexpected problems, the yield components were not estimated during 2012.

2.3.2 Correlations between physiological, yield and morphological traits

In the control treatment, kernel weight had a positive association with leaf wax but the main spike kernel number did not show any correlation whereas, in heat treatment both kernel number and weight showed no association with wax. Kernel weight was negatively correlated with leaf temperature in control treatment. Further, the negative correlation of kernel number with leaf and spike

temperature in both heat and high temperature treatments indicates the adverse effect of temperature on yield (Table 2). The positive association of kernel weight and kernel number with stomatal conductance and fluorescence suggests that those are indirect estimator of plant health for photosynthesis. Leaf (r= -0.13) and spike temperature (r =-10) showed a negative correlation with wax indicating their role in reducing CT. Further, the leaf and spike temperature showed negative correlation with stomatal conductance in both treatments suggesting that the increase in leaf conductance decreases canopy temperature (Table 2).

The flag leaf and spike length had a positive correlation with yield components, indicating their possible role in yield gain in both treatments. In control treatment, a negative association between spike length and leaf temperature was observed. In addition, the flag leaf length had a significant negative association with spike length (r=-0.11 and r=-0.15) in both treatments indicating that the leaf length may not directly influence yield by producing longer spike rather it may increase the number of spikelets or grains per spike to contribute to yield gain. However, the leaf length had positive relation with peduncle length but spike length was negatively correlated with peduncle length (Table 2). The negative correlation between spike and peduncle length may be due to the dwarfing gene that reduces peduncle length in order to increase grain number in the spikes.

Table 2 Pearson's correlation analysis of epicuticular wax (EW), stomatal conductance (SC), chlorophyll fluorescence (FL), leaf temperature (LT), spike temperature (ST), flag leaf length (FLL), spike length (SL), peduncle length (PL), kernel weight (KW) and main spike kernel number (Mkn) for plants under control and high temperature treatment

	Mkn	**LT**	**ST**	**SC**	**FL**	**FLL**	**SL**	**PL**	**EW**
Kw	0.22***	-0.27***	-	0.18**	0.16**	0.15**	0.10*	-	0.17**
Mkn		-0.31**	-0.36**	0.47***	0.10*	0.18**	0.22***	-	
LT			0.30**	-0.10*	-	-	-0.23**	-	-0.13*
ST				-0.40***	-	-	-	-	-0.10*
SC						-0.18***	-	-	
FLL							-0.11*	0.13*	-
SL								-0.23**	0.11

	Mkn	**LT**	**ST**	**SC**	**FL**	**FLL**	**SL**	**PL**	**EW**
Kw	0.29***	-	-	-	-	0.14**	-	0.15**	-
Mkn		-0.14*	-0.28*	-0.12*	-	0.14**	0.30***	0.17***	-
LT			0.52***	-0.13*	-0.24**	-	-	-	-0.15*
ST				-0.16**	-0.15**	-	-	-	-0.13*
FLL					-	-	-0.15**	0.28***	-
SL								-0.24**	-

*, **,*** significant at p<0.05, 0.01, 0.001 respectively

2.4 Discussion

The leaf wax content varied significantly between the genotypes and ranged from 1.33 to 8.52 in the year 2012 and 3.0-9.2 mg/dm^2 in 2013 in control and 2.12 to 9.26 mg/dm^2 and 2.8 to 10.5 mg/dm^2 in high temperature treatment in 2012 and 2013 which was considerably greater than the reported values for wheat and other crops (Ebercon et al. 1977; Cruz et al. 1983; Uddin et al. 1988). The

differences among the lines for leaf wax could be due to the modulation of enzyme activities associated with the wax biosynthesis and transport of waxes to the epidermis by protein carriers or the mutation of genes involved in wax production and transportation (Samuels et al. 2008). Further, the environmental factors such as temperature, light and humidity also causes variation in the wax content.

Negative correlation of leaf and spike temperature with wax and stomatal conductance (Table 2) suggests that the wax content reduces the leaf temperature and stomatal conductance to make the canopy cooler. Stomatal conductance is affected by leaf properties such as wax deposition and wax structure, which ultimately determines the hydraulic permeability of the leaf (Shepherd and Wynne Griffiths 2006). Glaucous leaves decreased leaf temperature and stomatal water loss in order to reduce gas exchange (Clarke et al. 1988) and it was found that stomatal conductance was lower in glaucous leaves than non-glaucous under dry land conditions (Johnson et al. 1982). The negative association of stomatal conductance with leaf and spike temperature suggests that SC act as an alternative mechanism to reduce heat load and increase canopy cooling.

The reduced kernel number in the primary spike in response to short term heat stress supports the previous findings that yield components in wheat are sensitive to high temperature stress (Ferris 1998). The negative association of leaf and spike temperature with yield components supports the findings that a strong correlation exists between canopy temperature and yield (Reynolds et al. 1998).

Plant organs show different temperatures relative to their position on the plant due to differences in light energy absorption and reflectance. The finding that there was no significant difference between the parental cultivars for the traits is likely due to the duration and intensity of high temperature stress were probably not enough to identify the differences.

The positive association of flag leaf and spike length with yield components confirmed the observation that breeding for certain morphological trait would be a helpful criteria in increasing the yield (Gardener et al. 1966) and the results were in accordance with the findings of others (Simpson 1967). The relation between spike length and kernel weight and number shows an increase in spike length accommodates more spikelets, which in turn increase the kernel number and yield (Hsu and Walton 1971). However, the negative correlation between flag leaf length and spike length is due to the fact that both flag leaf and spike grow simultaneously and there will be a competition for carbohydrate reserve (Villegas et al. 2007). In addition, the spike length showed a negative correlation with peduncle length. This is due to the *Rht* dwarfing alleles that results in reduced cell length and width and ultimately peduncle length. The reduced peduncle length liberates more assimilates partitioned to the spike, thereby, allowing the distal florets to undergo fertilization and thus increase in grain number (Rebetzke et al. 2011).

2.5 Conclusion

The greenhouse study on TAM 112 x TAM 111 RIL showed variation for leaf wax and other physio-morphological traits indicating there is transgressive segregation for the traits. The association between wax and leaf temperature indicates its possible role in protecting the leaf from high temperature stress by increased reflectance. The negative association of stomatal conductance and fluorescence with canopy temperature suggests that the higher stomatal conductance is associated with cooler canopies, which provide an avoidance type of heat resistance during high temperatures stress. The negative relation with fluorescence is due to reduced chlorophyll content under high temperature conditions. In this study, leaf wax didn't show any association with neither conductance nor fluorescence. The grain yield is determined by several components and the correlation between leaf and spike length with yield components suggests their contribution to yield and should be taken into account while selecting varieties for higher yield.

3. QTL MAPPING OF EPICUTICULAR WAX, AND ITS EFFECT ON CANOPY TEMPERATURES AND YIELD COMPONENTS IN TAM 112 X TAM 111 RECOMBINANT INBRED LINES UNDER CONTROLLED AND WATER DEFICIT CONDITIONS

3.1 Introduction

Wheat (*Triticum aestivum*) is the most widely consumed cereal crop in the world. Though wheat best adapts to cool weather conditions, it is grown in a wide range of environments that include temperate, subtropical and tropical regions. Although wheat production is feasible in warmer areas, heat stress is a common occurrence especially during anthesis and grain filling (Reynolds et al. 1994). Temperature of 35°C for a brief period can drastically reduce yield due to early senescence and restrict carbon assimilation (Punia et al. 2011). Further, the damage due to heat stress is often compounded by water stress and both severely limit crop productivity. It is estimated that the drought affects 50% and 70% of wheat area under production in developing and developed nations respectively (Kirigwi et al. 2007). However, discerning heat and drought stress is complex due to its quantitative inheritance and interaction with environment, thus making it difficult to understand the genetic basis of these traits (Saint Pierre et al. 2012). In addition, biotic stresses also significantly reduce yield in many wheat-growing regions (Liu et al. 2014). However, plants adapt to heat and drought stress using number of defined physio-morphological

and molecular mechanisms. Different traits that are reported to correlate with high temperature and drought stress are root, height, awns, osmotic adjustment, canopy temperature, abscisic acid (ABA) and glaucousness (Trethowan et al. 2002; Quarrie et al. 2005; Olivares-Villegas et al. 2007; Bennett et al. 2012; Tsilo et al. 2013).

In a complex genome like hexaploid wheat, identification of quantitative trait loci (QTL) governing abiotic and biotic traits will enhance breeding wheat varieties for different environments. Since yield is the most important trait, most QTL for stress tolerance are determined through yield and yield components under heat and drought stress conditions. However, yield itself is controlled by a large number of QTL with both major and minor effects (Cuthbert et al. 2008). In addition, yield and yield components are complex traits influenced by environment and large genotype x environment interactions (GxE) (Kirigwi et al. 2007). Further, identification of QTL is complicated by QTL x E interactions (QEI) for complex traits. Due to the QEI, the QTL detected in individual environments were not detected across environments in wheat (Campbell et al. 2003). Approximately 2 to 15% of phenotypic variations are accounted by QTL associated with yield and yield related traits (Quarrie et al. 2005; McIntyre et al. 2010). Traits such as early flowering, grains per spike, harvest index, stem carbohydrate and grain number are known to be associated with higher yields (McIntyre et al. 2010). QTL for various traits in wheat have been identified across genome. For example, QTL for grain yield and other agronomic traits including grain weight, 1000 kernel weight, kernels per spike and spike per square meter were identified in chromosome 3A

(Mengistu et al. 2012). A major QTL for grain yield and yield components including grain fill rate, spike density, grain m^{-2}, biomass production and drought susceptibility index (DSI) was identified on chromosome 4AL (Kirigwi et al. 2007). In another study, five QTL associated with grain yield were identified on chromosomes 2A, 2D, 3B and 6A. In a Chinese wheat population, 99 putative QTL for nine traits associated with grain yield were identified. The pleiotropic effects of yield were detected on chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 4B, 4D, 5B, 6D and 7D (Wang et al. 2009).

In wheat, canopy temperature (CT) and canopy temperature depression (CTD) are associated with grain yield under stress conditions and have been shown to co-localized with yield and yield component QTL (Mason et al. 2011). QTL associated with CT and grain yield have been identified in chromosomes 1B, 2B, 3A, 3B, 4A, 5A, 5B, and 7A (Pinto et al. 2010; Bennett et al. 2012). In another study, QTL for CTD have been identified on chromosomes 2D, 3B, 5A, 5D, 6D and 7A (Mason et al. 2011; Mason et al. 2013). Further, studies have found association between CT and leaf epicuticular wax in reducing canopy temperature.

Leaf glaucousness is considered reliable trait under water deficit conditions and is determined by the gene W1 located on chromosome 2BS, whereas the homoelogous loci for wax production and inhibition are on chromosome 2DS. High temperature and drought stress significantly influence the amount of epicuticular wax resulting in increased deposition on leaf surfaces (Richards et al. 1986; Kim et al. 2007). A spike glaucousness gene in wild emmer was mapped on chromosome 1BS, whereas in durum

wheat, wax production gene was detected on chromosome 2A (Liu et al. 2006).

In wheat, QTL for flag leaf glaucousness were mapped on chromosome 1D, 2B, 2D, 3A, 4D, 5A, 5B, and 6A (Mason et al. 2010; Bennett et al. 2012). In rice, two QTL for leaf epicuticular wax were detected on chromosome 3 and 8 explaining 22.9% and 9.6% of phenotypic variation respectively (Srinivasan et al. 2008). Though QTL associated with pest, disease and wheat quality have been used in breeding programs (Liu et al. 2014), QTL linked to epicuticular wax are relatively few. The objective of this study was to identify QTL for epicuticular wax and its association with reduced canopy temperatures and yield stability in TAM 112 x TAM 111 RIL population grown under controlled and limited water conditions in the field.

3.2 Materials and methods

3.2.1 Plant materials and field trials

The study was conducted in a set of 124 recombinant inbred lines (RILs) derived from a cross between TAM 112 and TAM 111 in the year 2012 and 2013. Both TAM 112 and TAM 111 are hard red winter wheat varieties developed by the Texas Agricultural Experiment Station (TAES).

The pedigree of TAM 112 is U1254–7-9–2-1/TXGH10440 whereas TAM 111 is TAM107’//TX78V3630/‘Centurk78’/3/TX87V1233. TAM 112 is adapted to the low-rainfall areas of the Southern Great Plains of the U.S and has superior grain and forage production and suitable for dryland and limited irrigation. TAM112 is resistant to wheat

curl mite and greenbug, carries gb3 gene from TXGH10440 but susceptible to rust. TAM 111 is a semi-dwarf, high yielding variety, resistant to stripe rust and well suited for dryland production of high plains of Texas (Rudd et al. 2014). For this study, F6-derived RILs were used in the 2012 and 2013 experiments.

The trials were conducted at Uvalde, College Station, Chillicothe, Bushland and Etter, Texas in 2012 and 2013. In Uvalde and College Station there were two treatments, control and water-deficit, with 2 replications in each environment. Both control and water-deficit treatments received the same amount of irrigation until the stem elongation stage (Feekes 5), at which point irrigation was stopped for the water-deficit treatment. The amount of water supplied for control and water-deficit were 375 mm and 175 mm, respectively. Due to volunteer mix in College Station and uneven stand in Etter the plots were not harvested in the year 2012. Again, due to uneven stand in Uvalde and freeze damage in Chillicothe the plots were not harvested for yield calculation in the year 2013.

3.2.2 Wax quantification

Four leaf discs of 0.8 cm in diameter samples from flag leaf were collected for wax analysis at 10 days after pollination (DAP) using paper punches. Leaf wax was extracted and quantified using colorimetric technique as described (Ebercon et al.1977). Leaf wax was extracted with 1ml HPLC grade chloroform for 30 seconds, transferred to a clear 1.8 ml glass gas chromatography vials (GC) (VWR Auto sampler

Vial) and vacuum dried. The dried extract was oxidized with 300 µl acidified potassium dichromate ($K_2Cr_2O_7$) and heated for 30 minutes (min) at 100 °C in a water bath. Later, 700 µl of deionized water was added to the vials and allowed to cool for the color to develop. Thereafter, 100 µl samples were loaded in 96 well clear polystyrene plates (Greiner Bio-One, NC, USA) and were analyzed using spectrophotometer (PHERAstar plus, BMG LABTECH) to determine the optical density at 590 nm. Following the same extraction procedure, a standard curve was plotted for some randomly selected flag leaves and the resulting wax-chloroform was used for the serial dilution technique. The standard curve was used to calculate the wax levels based on leaf area.

3.2.3 Canopy temperature and yield measurements

Flag leaf and spike temperatures were taken approximately at ten DAP using a handheld infrared thermometer. The thermometer was held at 45 degree angle to the leaf and spike and the measurements were taken between 12 pm to 3 pm. Leaf temperature was taken in the years 2012 and 2013, whereas spike temperature was recorded only in 2013. Due to the fluctuation in weather conditions, canopy temperature was not recorded at some of the field trials.

To estimate the yield components, fifty random heads were collected from each plot at maturity. The yield components kernel weight (KW), kernel number (KNO) and spikes per meter squared (Spm^2) was calculated using single head weight and plot yield. Each plot was harvested using a combine harvester to measure grain yield per plot.

3.2.4 Statistical analysis

All data was analyzed using statistical software SAS (SAS v9.2). PROC CORR (Pearson's correlation method) was used to analyze the trait correlations and PROC GLM model was used variance test analysis. A test for normality was done for each of the traits across individual locations and years, followed by combined analysis.

3.2.5 QTL analysis

DNA was extracted from the leaf tissue of both parents and RILs and was genotyped at the USDA- ARS, Fargo, North Dakota. High throughput genotyping of 90,000 (90K) SNP (single nucleotide polymorphism) was performed (Infinium iSelect array from Illumina). The 90K SNP clustering and annotation was performed using Genome Studio v2011 (Illumina). The 3166 markers identified as polymorphic were used to construct linkage groups using JoinMap 4 software with regression mapping method and Kosambi mapping function. The resultant linkage groups were used to detect QTL using MapQTL 6 software. Multiple QTL Mapping (MQM) analysis was conducted across environments to detect main effect QTLs. For linkage group construction, a significance level of 0.05 was set and for QTL mapping, 10000 permutations were used to determine the maximum likelihood of odds (LOD) score threshold. A QTL was determined to be present, if the LOD score is 2.5 and above and considered to be stable, if present in at least two environments. Graphical representation of QTLs was performed using MapChart 2.2 software.

Table 3 Field environments of the RIL population grown in 2012 and 2013

Locations		Abbreviation	Treatments	Traits measured
Year -2012	**Year - 2013**			
College Station, TX	College Station, TX	CS	Irrigated and limited irrigated	Wax, LT, ST, KNS, KW, Yield and Spm
Uvalde, TX	Uvalde, TX	UV	Irrigated and limited irrigated	Wax, LT, ST, KNS, KW, Yield and Spm
Bushland, TX	Bushland, TX	BD	Limited irrigation	Wax
Etter, TX	Etter, TX	ET	Irrigated	Wax, KNS, KW, Plot yield
	Chillicothe, TX	CH	Limited inrigation	Wax, KNS and KW

Wax – leaf and glume wax, LT- Leaf temperature, ST- Spike temperature, KNS- Kernel no/spike, KW – Kernel weight, Spm – Spike/m^2. ST was taken only in 2013. ET 2012 – only wax was measured

3.3 Results

This study was conducted in different locations across Texas. The field locations, treatments and the traits measured were summarized in Table 3. The parent cultivars TAM 112 and TAM 111 showed no significant differences for wax content in both control and water deficit treatments across locations in the years 2012 and 2013 (Fig.1). Similar results were observed for glume wax (data not shown) in the parental cultivars. Among the irrigated trials across locations in the two years 2012 and 2013, CS produced higher grain yield than other locations, whereas in water deficit experiments, Uvalde had higher yield than BD. Though the yield of TAM 112 was higher than TAM 111 in few locations, the difference was not statistically significant (Fig.2).

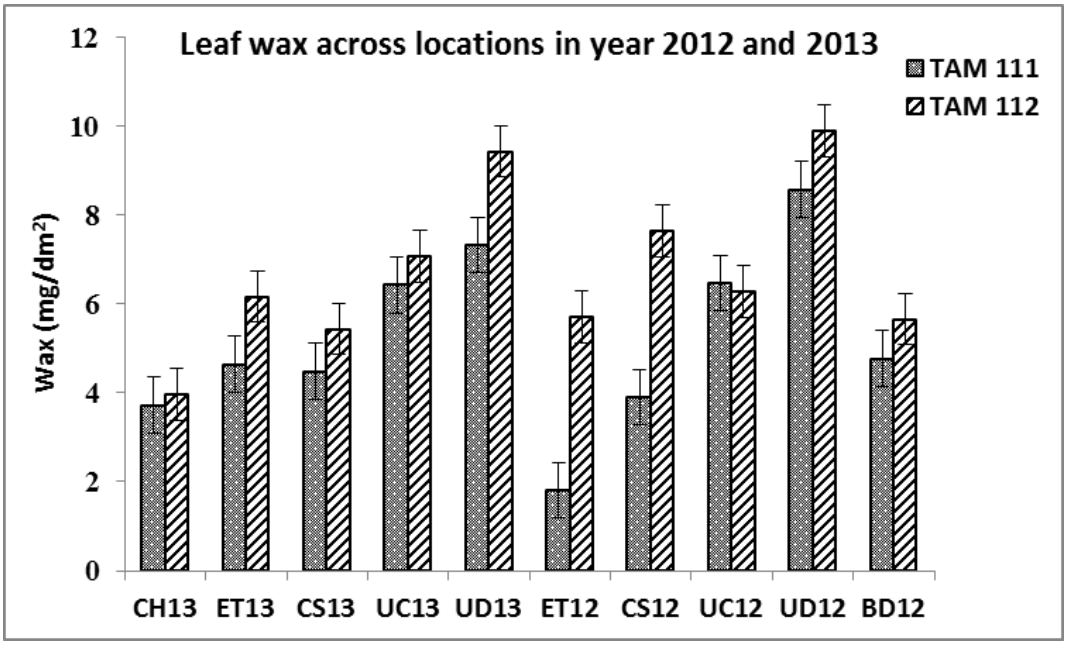


Fig.1 Leaf wax content in different locations in years 2012 and 2013 between the parents TAM 112 and TAM 111 (CH-Chillicothe, ET-Etter, CS-College Station, UC-Uvalde, BD-Bushland; C-Control and D-Drought; 12-year 2012 and 13-2013)

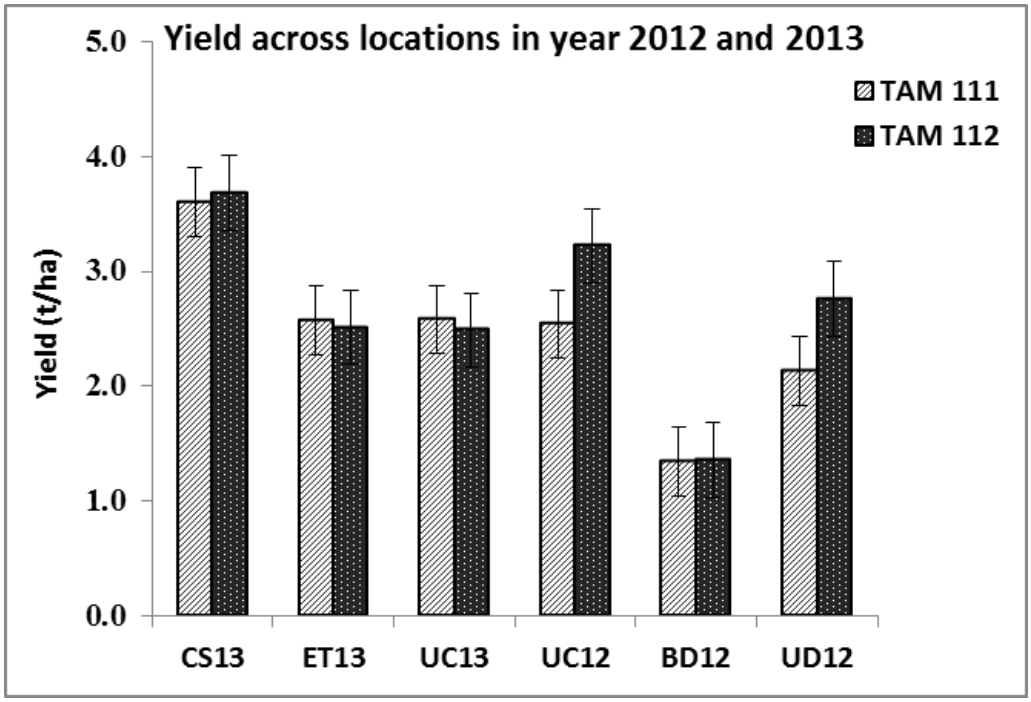


Fig.2 Grain yield between TAM 112 and TAM 111 across locations in years 2012 and 2013 (CS-College Station, ET-Etter, UC-Uvalde, BD-Bushland; UC-Control and UD-Drought; 12- year 2012 and 13 year 2013)

The combined analysis of variance for all the traits was performed to determine the effects of the RIL (G), environment (E), and genotype by environment interactions (GxE). The combined analysis showed that the RIL population responded differently to diverse environments. The genotypes were significant for all the traits. The effect of environment can be derived from high G x E effect particularly for wax and spike/m^2 (Table 4).

Table 4 Combined analysis of variance (ANOVA) of RILs for yield, kernel number (KNS), kernel weight (KW), spike per meter square (spike/m^2), leaf wax, glume wax and leaf (leafT) and spike temperature (spikeT) across different environments

		Mean squares			
Trait	**Unit**	**Genotype (G)**	**Env (E)**	**GXE**	**Rep (Env)**
Yield	tons/ha	0.42***	59.43***	0.27*	19.02***
KNS	no	60.65	7865.12***	20.21	448.45***
KW	g	0.00***	0.007***	0.00**	0.00**
Spike m^{-2}	no	5573.34***	73730.18***	3818.34***	36395.00**
Leaf wax	mg/dm^2	21.94***	1198.86***	29.58***	84.94**
Glume wax	mg/dm^2	12.72	1005.74***	9.65	372.53***
LeafT	°C	3.83***	2439.40***	2.19*	35.10***
SpikeT	°C	4.21***	4407.61***	2.37***	74.93***

*, **,*** significant at p<0.05, 0.01, 0.001 respectively

The range values for the parents and RIL are presented in Table 5. Though the maximum yield in control environments for cultivars TAM 112 and TAM 111 were little higher than drought environments, there was no significant differences. Among the RIL, grain yield varied between 0.81-4.13 tons ha^{-1} in water deficit environment and 1.14 to 4.62 tons ha^{-1} in irrigated trials across locations. Kernel number for RIL ranged from 7 to 47 in drought whereas 11 to 59 in control experiments. The number of spikes per meter squared varied between 136 to 520 and 89 to 576 in drought and control experiments respectively. Among the parents TAM 112 and TAM 111 there was difference in control and drought treatments, as expected. In control, TAM 112 had number of spikes up to 446/m^2 whereas in drought it ranged from 279-388/m^2. In TAM 111, the numbers of spikes were lesser than TAM 112 and it ranged from 186-263 in drought and 191-440 in control treatment. Leaf wax in the RIL population ranged from 0.88 mg dm^{-2} to 37.29 mg dm^{-2} across drought environments and 0.47 – 36.28 mg dm^{-2} across control environments (Table 5).

In drought and irrigated experiments, the parental cultivar TAM 112 produced maximum of 11.10 mg dm^{-2} and 8.86 mg dm^{-2} of leaf wax, and 10.41 dm^{-2} and 9.27 mg dm^{-2} glume wax respectively. In control trials, TAM 111 produced 9.23 mg dm^{-2} leaf and 9.51 mg dm^{-2} of glume wax, whereas TAM 112 had 8.86 mg dm^{-2} and 9.27 mg dm^{-2} leaf and glume wax respectively (Table 5). Leaf and spike temperature among the RIL in drought environment ranged from 26.83 -44.4°C and 29.50-41.90°C respectively.

Table 5 Physiological and phenological trait ranges for the parents TAM 112, TAM 111 and RIL population grown under drought and irrigated environments across locations

Traits	Drought			Control		
	TAM 112	TAM 111	Range	TAM 112	TAM 111	Range
Leaf wax (mg/dm^2)	1.70 – 11.10	1.84 – 10.70	0.88-37.29	2.71 – 8.86	2.04 – 9.23	0.47-36.28
Glume wax (mg/dm^2)	4.43 – 10.41	3.75 – 10.38	1.38-27.54	1.91 – 9.27	1.86 – 9.51	1.15-28.33
Yield (tons/ha)	1.30 – 3.63	1.27 – 3.39	0.81-4.13	2.09 – 3.85	1.82 – 4.40	1.14 - 4.62
KNS (no)	9.0 – 35.0	10.0 – 37.0	7.0 – 47.0	20.0 -42.0	14.0 – 39.0	11.0 – 59.0
KW (g)	0.01 – 0.04	0.02 – 0.04	0.01-0.04	0.02 -0.04	0.02 – 0.04	0.02 -0.06
Spike/m^2 (no)	279 - 388	186.– 263	136 -520	245 - 446	191 - 440	89 - 576
Leaf temperature (°C)	30.4 -40.9	28.1– 43.4	26.83 - 44.40	25.0 – 34.4	25.7 – 34.9	22.45-36.2
Spike temperature (°C)	36.0 - 38.9	31.5 – 37.0	29.50 - 41.90	26.0 – 31.6	25.4 – 31.3	25.10-33.60

To identify the association between wax, CT and yield components correlation analysis was done for each environment separately for the year 2012 and 2013. In CS, 2013 environment, Leaf wax was positively correlated with KNS (r=-0.13) but negatively associated with spike/m^2 (r=-0.11). However, glume wax was significantly correlated with KW (r=0.14) and yield (r=0.11). Grain yield was positively correlated with the yield components KW and spike/m^2 (r = 0.24 and r = 0.75 respectively) but KW showed negative association with KNS (r=-0.24) so as the KNS with spike/m^2 (r=-0.53) (Table 6). In ET 2013 and UC 2013 environment both leaf and glume wax did not show any association with yield and yield components. Similar to CS 2013, KW was negatively correlated with KNO in both ET and UC. Further, in UC environment yield showed negative correlation with KW (r=-0.12) but positively associated with KNS and spike/m^2. In UC 2012 environment, leaf wax negatively correlated with KNS but did not any association with SPM and yield whereas in UD 2012, yield and yield component was negatively correlated (r=-0.44 and -0.19) with both leaf and gluwax except gluwax with KNS. In addition, in both environments KNS was negatively correlated to spm (r=-0.58 and r=-0.43) (Table 6). Further, in UD 2012, LT showed negative association with yield and yield components. In all environments spm showed positive correlation with yield. For some of the locations like CH 2013 and ET 2012 yield data was not taken so as spike temperature to interpret the association of with yield and spike/m^2.

Table 6 Pearson's correlation of physiological and phenological traits measured for the TAM 112 x TAM 111 RIL population grown under different environments and years

CS 2013

Traits	Gluwax	LT	ST	Kw	Kns	Spm	Yield
ST (°C)	-	0.76***	-	-	-	-	-
Lfwax (mg/dm^2)	0.19**	-	0.13*	-	0.13*	-0.11*	-
Gluwax (mg/dm^2)		-	-	0.14*	-	-	0.11*
Kw (g)					-0.24***	-	0.24***
Kns (no)						-0.53***	-
Spm (no)							0.75***

UC 2013

Traits	ST	Kw	Kns	Spm	Yield
LT (°C)	0.69***	-	-	-	-
ST (°C)		-0.23***	0.28***	0.17***	0.23***
Kw (g)			-0.34***	-0.28*	-0.12*
Kns (no)				-	0.56***
Spm (no)					0.79***

ET 2013

Traits	Gluwax	Kns	Spm	Yield
Lfwax (mg/dm^2)	0.83***	-	-	-
Kw (g)		-0.41***	-0.12*	0.22***
Kns (no)			-0.56***	-
Spm (no)				0.47***

*, **, *** significant at p<0.05, 0.01 and 0.001 respectively

Table 6 Continued

UC 2012			
Traits	**Kns**	**Spm**	**Yield**
Lfwax (mg/dm^2)	-0.19*	-	-
Kw (g)	0.30***	-	0.34***
Kns (no)	-	-0.58***	-
Spm (no)	0.70***		

UD 2012				
Traits	**LT**	**KNS**	**Spm**	**Yield**
Lfwax (mg/dm^2)	-0.22*	-0.19*	-0.30***	-0.44***
Gluwax (mg/dm^2)	-	0.19*	-0.31*	-0.19*
LT (°C)	-	-0.17*	-0.20*	-0.35***
KW (g)		-	-	0.31***
KNS (no)			-0.43***	0.20*
Spm (no)				0.74***

The genetic linkage map was constructed with 3166 SNP markers. A total of 98 QTL were detected across environments for leaf and glume wax, canopy temperature and yield and yield components. The QTL were detected in chromosomes 1B, 2B, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 6A, 6B, 6D, 7A, 7B and 7D (Fig.3). Among the 98 QTL, 18 loci across 10 chromosomes were associated with leaf epicuticular wax. For glume wax 14 QTL were identified with QTL explaining 7.6 to 13.6% variation (Table 7). For leaf wax the QTL explained between 8-10.4% phenotypic variations. The LOD scores of above two were considered for wax QTL. For glume wax LOD score ranged from 2 to 3.89. Nine QTL were associated with canopy temperature, in which 5 QTL for spike temperature on chromosome 1B, 2A and 3A and 4 for leaf temperature QTL on chromosome 2A, 3B and 7D were detected (Fig.3).

Yield and yield components QTL were found across the chromosomes. For yield, the QTL explained about 8.7 to 12.3 % variation, whereas for yield components such as KWT it ranged 8.7 to 12.5% and KNO it was from 9.1 to 14.3% and spike/m2 it ranged from 8.6 to 16.7% phenotypic variation. There were about 15 QTL for KW, 19 QTL for KNO, 8 QTL for spike/m2 and 11 QTL for yield were identified. Both TAM 111 and TAM 112 were contributed favorable alleles for all the traits. TAM 111 associated with leaf wax at 7 loci located on chromosome 3A, 5B, 7B and 7D whereas TAM 112 contributed favorable alleles for 11 loci (Fig.4 and Table 7).

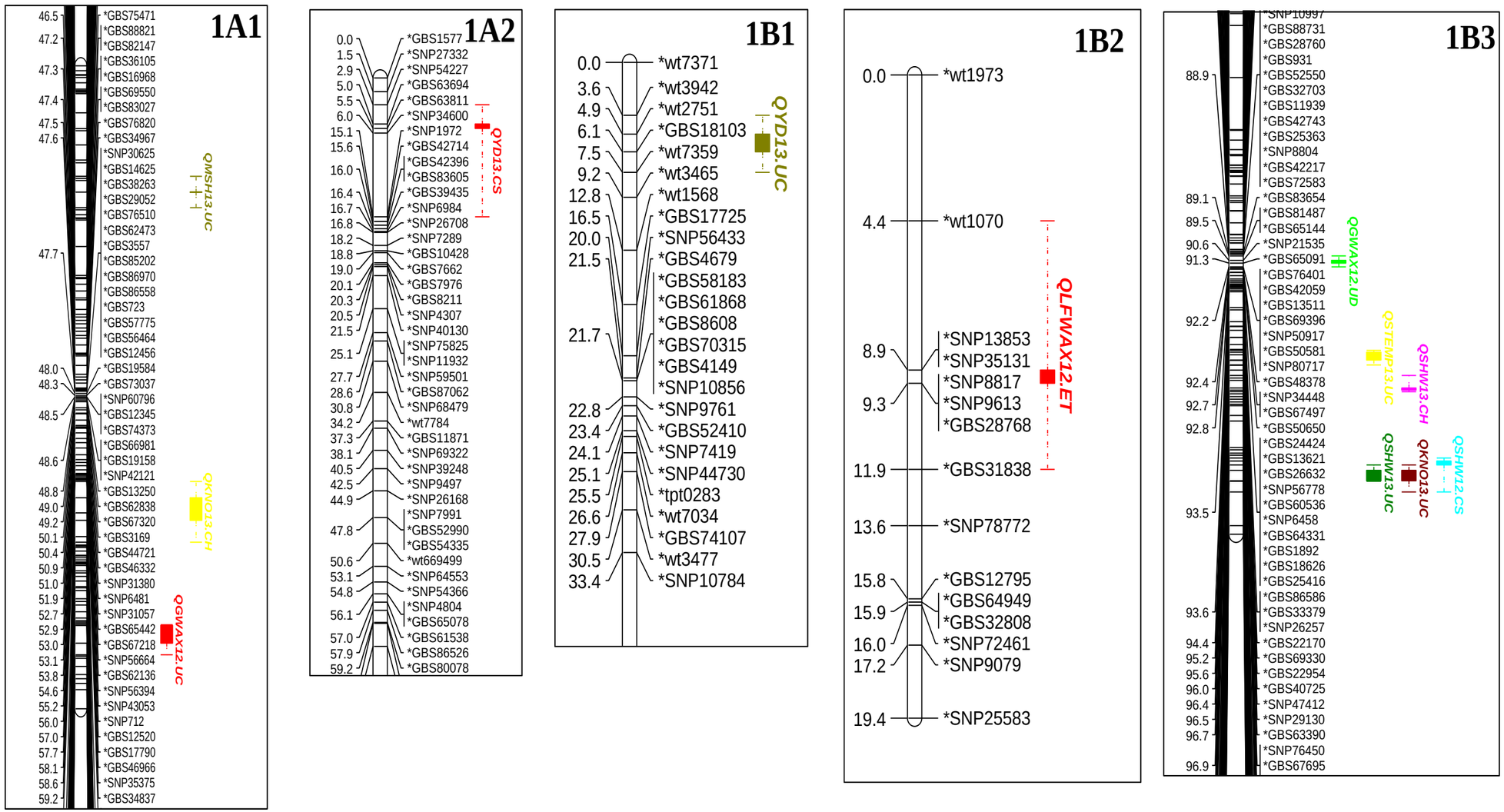


Fig. 3 Linkage map and quantitative trait loci for leaf and glume wax, leaf and spike temperature, grain yield, and yield components in the TAM 112 x TAM 111 RIL population across environments. (Marker positions are presented in cM)

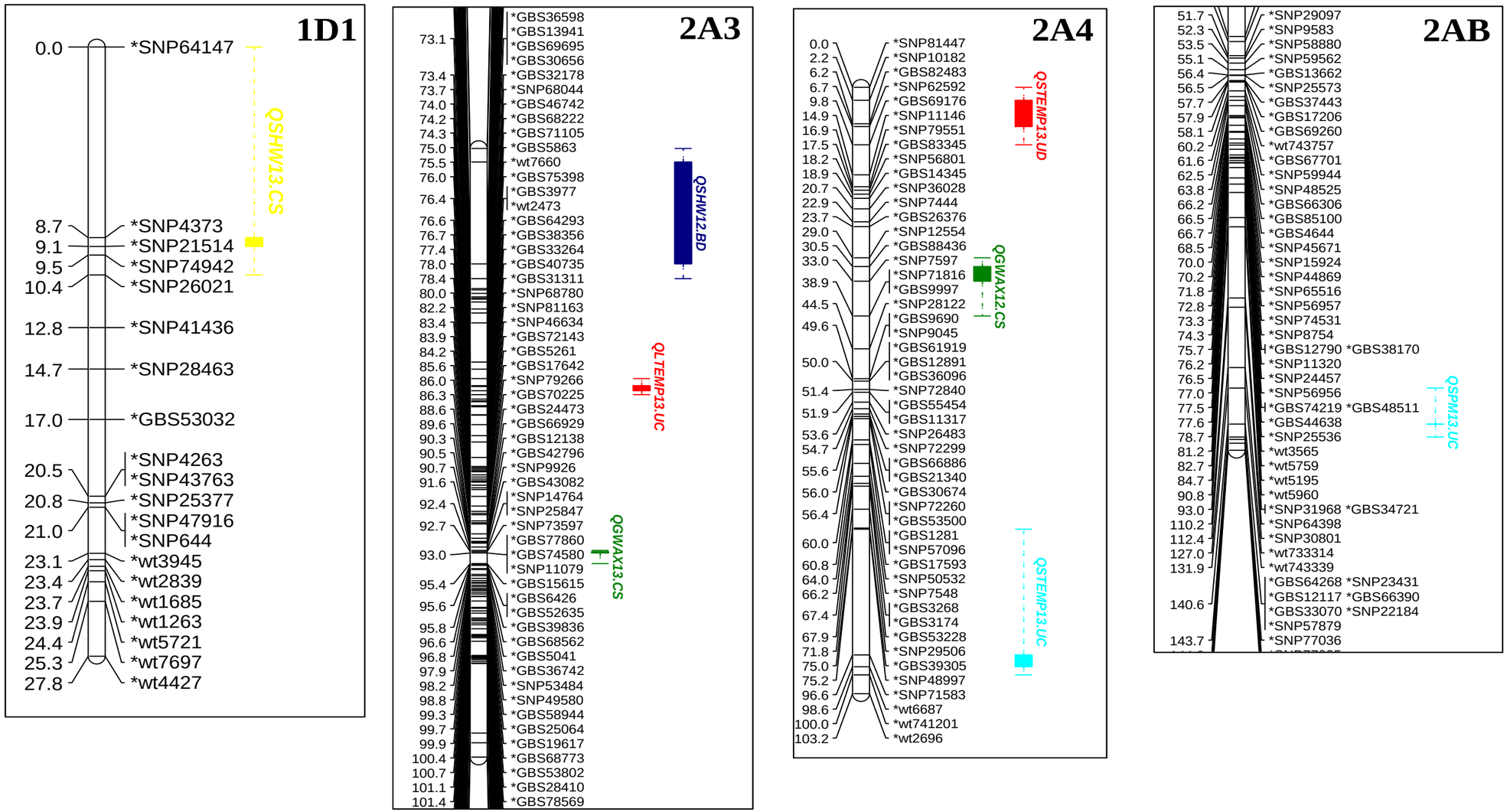


Fig.3 Continued

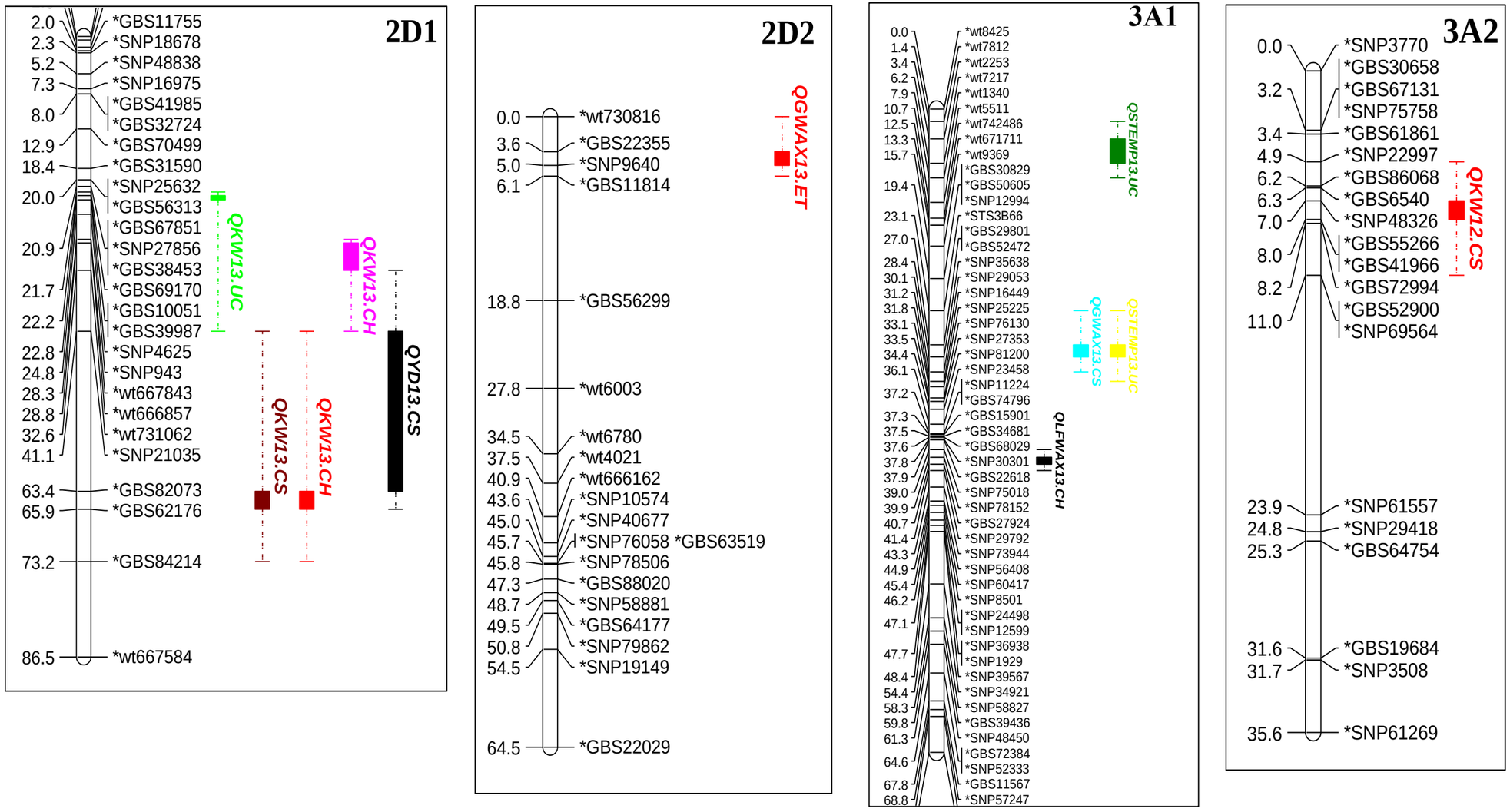


Fig.3 Continued

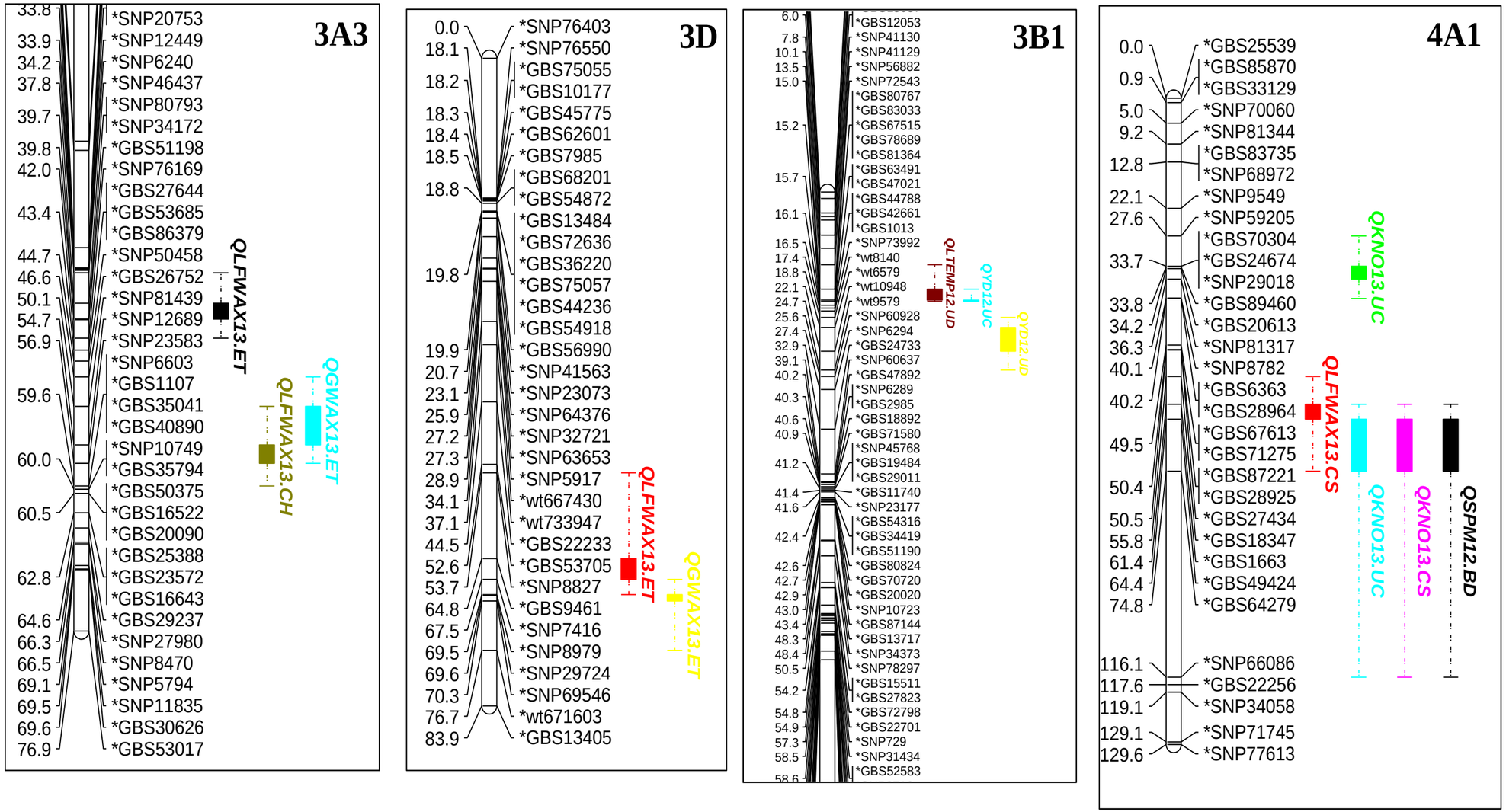


Fig.3 Continued

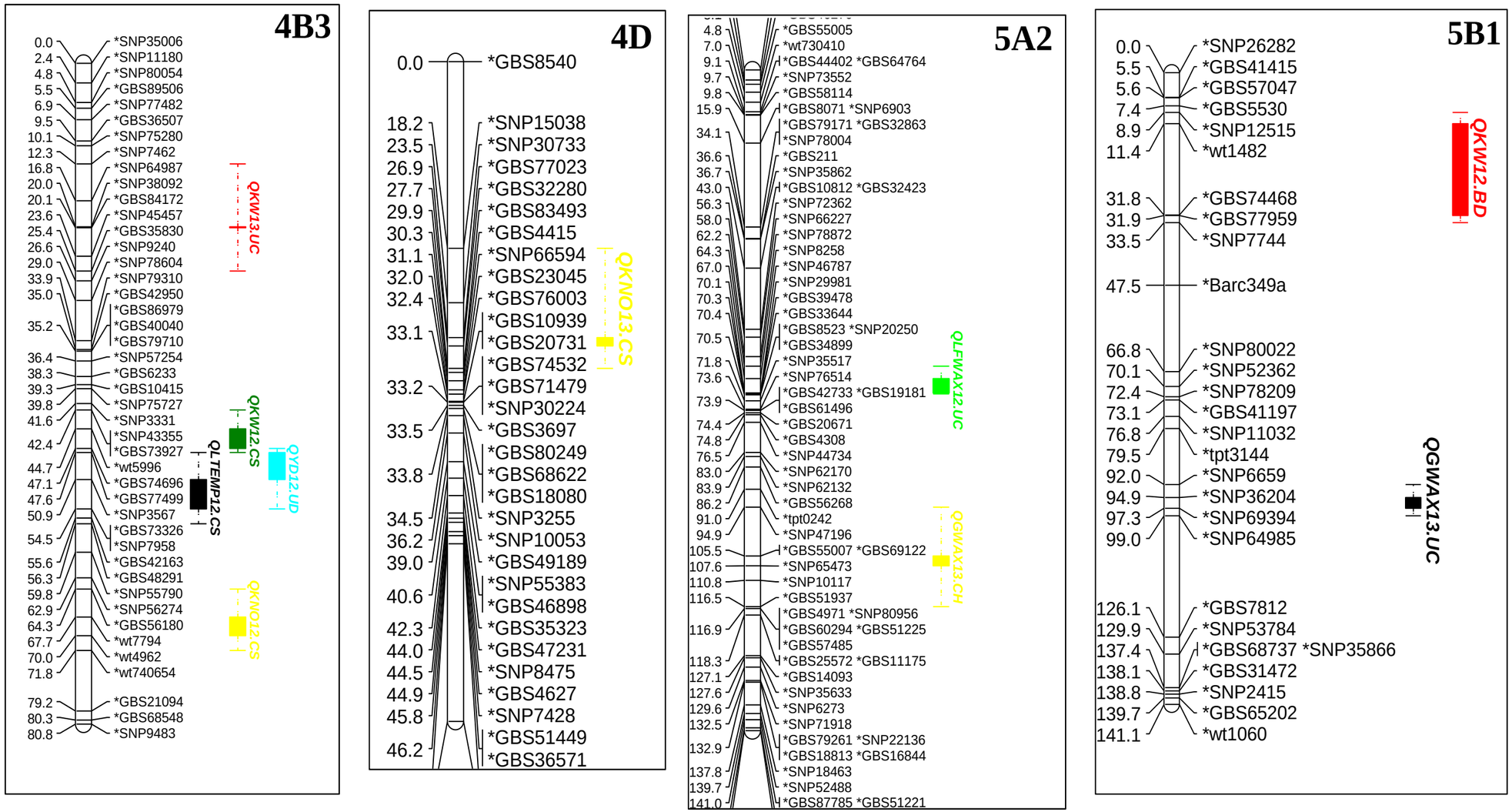


Fig.3 Continued

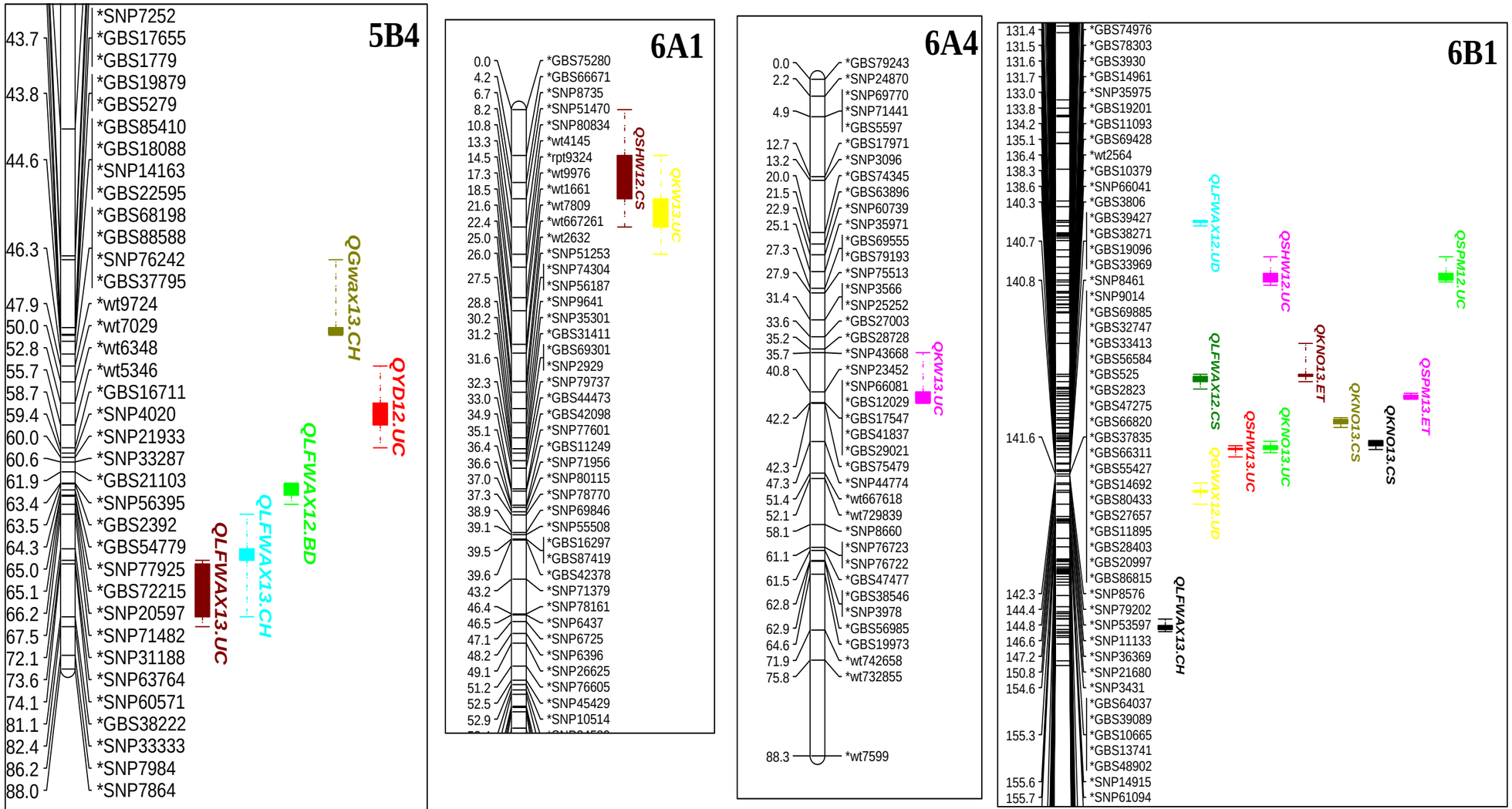


Fig.3 Continued

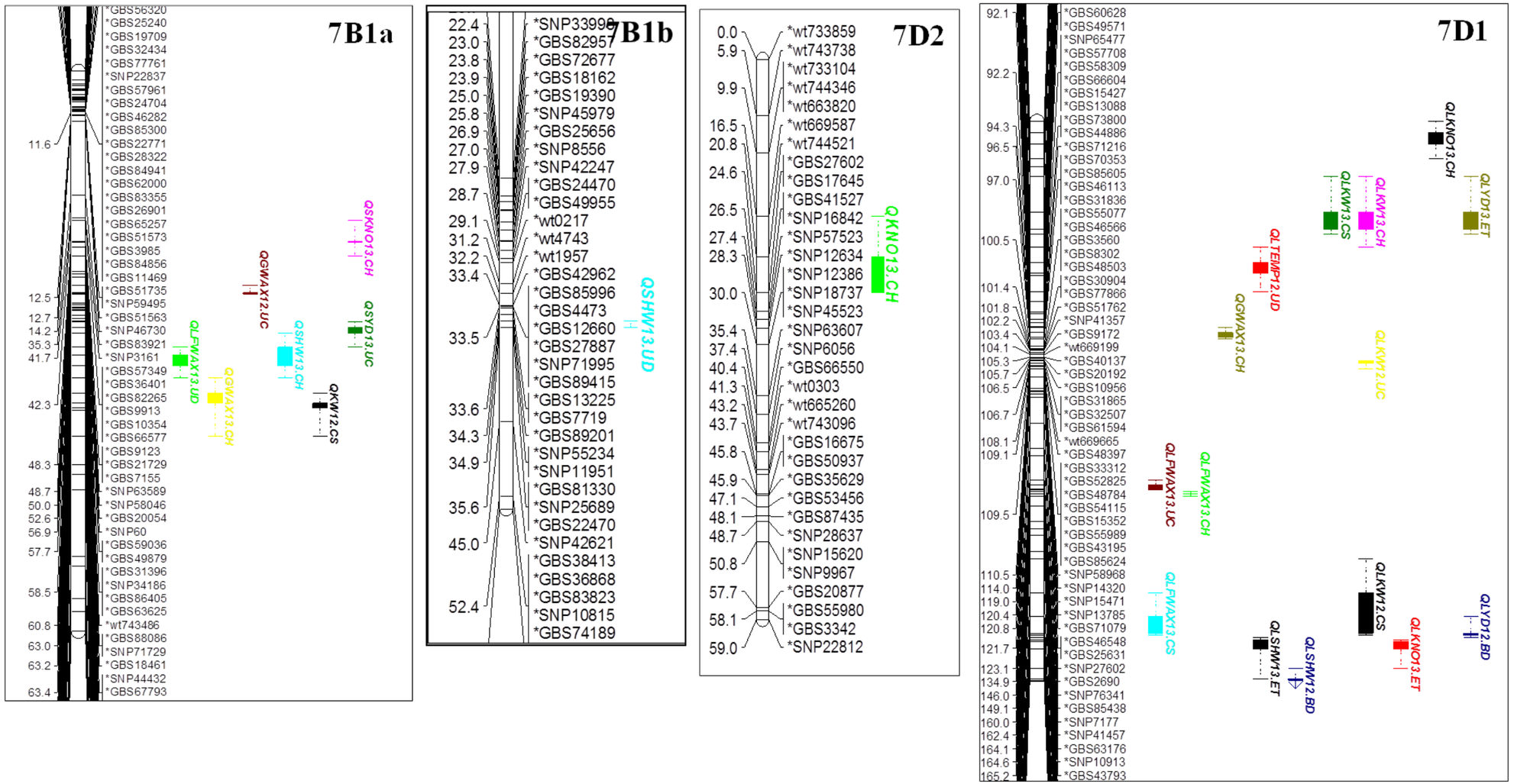


Fig.3 Continued

Table 7 Summary of QTL detected in the TAM 112 x TAM 111 populations for physiological and agronomic traits across different environments

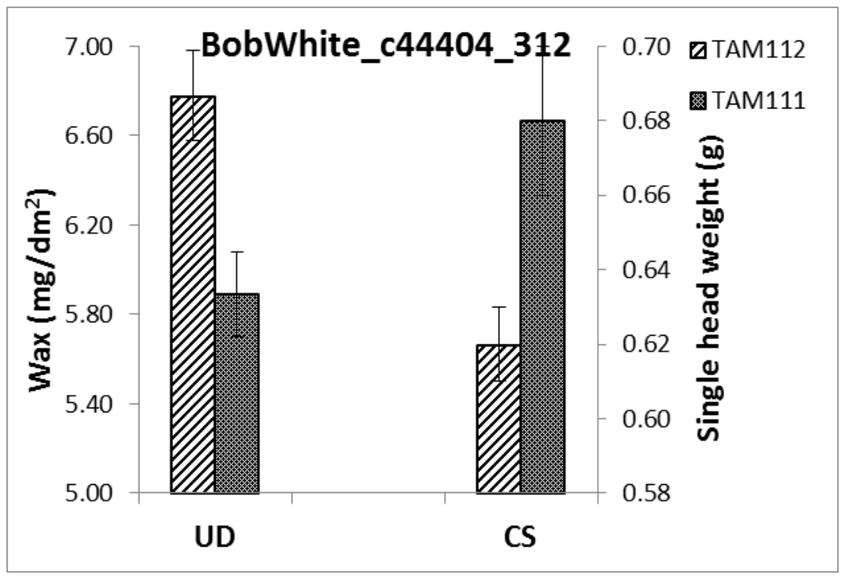
Traits	Location	QTL	Marker	Position	LOD	mu_A	mu_B	% Expl.	Additive	Favorable allele
Leafwax	UD13	QLFWAX.tam.7B.1a	BobWhite_c44404_312	83.692	2.07	5.88617	6.77667	8.8	-0.4452	TAM 111
	UC13	QLFWAX.tam.5B.4	GBS38222	81.122	2.7	7.9731	10.939	9.7	-1.483	TAM 111
	UC13	QLFWAX.tam.7D.1	Kukri_c15768_1383	162.37	2.3	7.82633	10.4885	8.4	-1.3311	TAM 111
	ET13	QLFWAX.tam.3A.3	wsnp_Ku_rep_c68484_6749982	39.72	2.45	5.46709	4.75311	8.7	0.35699	TAM 112
	ET13	QLFWAX.tam.3D	GBS9461	64.767	2.6	5.52931	4.78809	9.2	0.37061	TAM 112
	CS13	QLFWAX.tam.4A.1	GBS49424	64.374	2.25	4.70311	4.19789	8	0.25261	TAM 112
	CS13	QLFWAX.tam.7D.1	BobWhite_c5419_165	228.35	2.9	4.7529	4.1913	10.2	0.2808	TAM 112
	CH13	QLFWAX.tam.3A.1	wsnp_Ex_c44375_50444756	39.868	2.27	3.82896	4.36445	8.1	-0.2677	TAM 111
	CH13	QLFWAX.tam.3A.3	CAP11_c1464_135	54.747	2.42	4.35185	3.7949	8.6	0.27848	TAM 112
	CH13	QLFWAX.tam.5B.4	RFL_Contig1899_2863	73.623	2.52	3.87019	4.43881	8.9	-0.2843	TAM 111
	CH13	QLFWAX.tam.6B.1	GBS30053	188.993	2.74	4.48891	3.88552	9.7	0.3017	TAM 112
	CH13	QLFWAX.tam.7D.1	GBS41008	166.349	2.38	4.39741	3.84701	8.5	0.2752	TAM 112
	UD12	QLFWAX.tam.6B.1	GBS86295	64.886	2.97	14.2695	8.03515	10.4	3.11716	TAM 112
	UC12	QLFWAX.tam.5A.2	Kukri_c59306_675	67	2.09	6.86054	10.2117	7.6	-1.6756	TAM 111
	ET12	QLFWAX.tam.1B.2	CAP7_c1788_66	8.892	2.19	4.9411	4.14353	8.6	0.39878	TAM 112
	ET12	QLFWAX.tam.2BL	GBS35488	74.291	2.51	4.99879	4.15097	9.8	0.42391	TAM 112
	CS12	QLFWAX.tam.6B.1	Ku_c25908_277	113.532	2.14	13.9499	9.00668	9.2	2.47161	TAM 112
	BD12	QLFWAX.tam.5B.4	wsnp_Ex_c37410_45162932	64.951	2.18	5.38511	6.92775	8.1	-0.7713	TAM 111
Glumewax	UC13	QGWAX.tam.5B.1	IACX751	94.871	2.14	10.0832	11.3245	7.6	-0.6207	TAM 111
	ET13	QGWAX.tam.3A.3	wsnp_RFL_Contig2699_240252	50.094	2.59	13.4436	11.6408	9.2	0.90141	TAM 112
	ET13	QGWAX.tam.3D	Tdurum_contig28518_122	70.291	3.89	13.8695	11.641	13.4	1.11422	TAM 112
	CS13	QGWAX.tam.2A.3	GBS74580	93.012	2.48	10.7272	9.57439	8.9	0.5764	TAM 112
	CS13	QGWAX.tam.3A.1	IACX10917	28.43	2.16	9.5164	10.6096	7.8	-0.5466	TAM 111
	CH13	QGWAX.tam.5A.2	TA001900-1836	107.629	2.19	6.27559	7.40154	7.9	-0.563	TAM 111
	CH13	QGWAX.tam.5B.4	GBS17655	43.726	2.23	6.26972	7.40777	8	-0.569	TAM 111
	CH13	QGWAX.tam.7B.1a	GBS5528, GENE-4833_102	91.427	2.09	6.08238	7.19486	7.5	-0.5562	TAM 111
	CH13	QGWAX.tam.7D.1	GBS73800	94.304	2.13	7.22834	6.10535	7.6	0.5615	TAM 112
	UD12	QGWAX.tam.1B.3	Excalibur_c100908_482	90.612	2.02	8.31712	6.60126	7.9	0.85793	TAM 112
	UD12	QGWAX.tam.6B.1	BS00080544_51	146.562	3.58	6.34392	8.60076	13.6	-1.1284	TAM 111
	UC12	QGWAX.tam.1A.1	Excalibur_c75270_566	84.57	2.29	7.74186	5.36512	9.1	1.18837	TAM 112
	UC12	QGWAX.tam.7B.1a	GBS18461	63.183	2.65	7.79942	5.2419	10.4	1.27876	TAM 112
	CS12	QGWAX.tam.2A.4	GBS88436	30.463	2.37	4.15611	5.55545	11.3	-0.6997	TAM 111

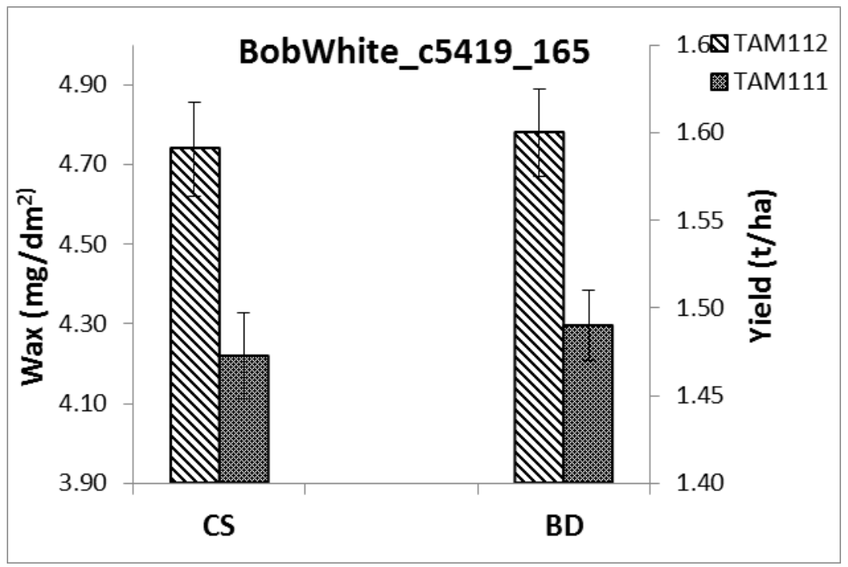
Table 7 Continued

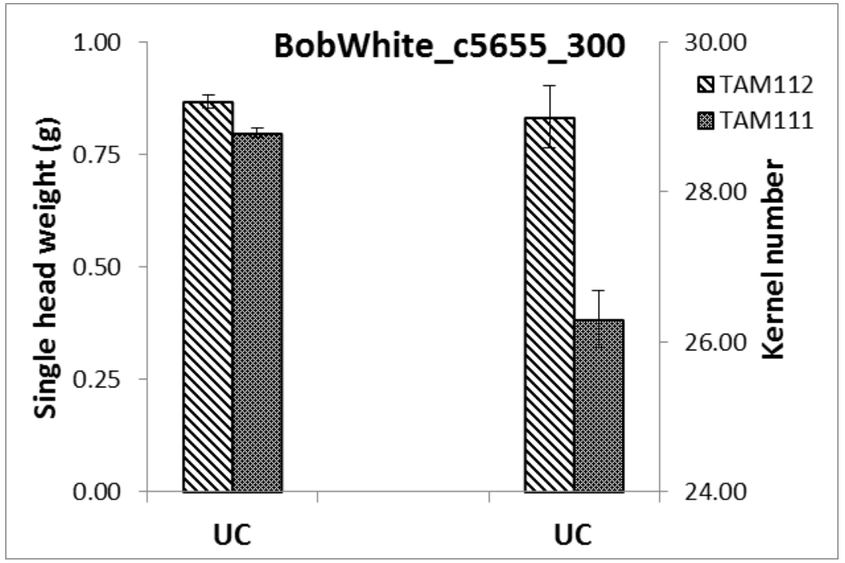
Traits	Location	QTL	Marker	Position	LOD	mu_A	mu_B	% Expl.	Additive	Favorable allele
LTEMP	UC13	QLTEMP.tam.2A.3	GBS61452	55.68	2.74	29.9049	29.2544	9.7	0.32522	TAM 112
	UD12	QLTEMP.tam.3B.1	RAC875_c33881_1227	13.542	2.03	30.4373	29.6807	7.3	0.37831	TAM 112
	UD12	QLTEMP.tam.7D.1	RAC875_c10636_525	67.675	2.53	30.4636	29.6202	9	0.42172	TAM 112
	CS12	QLTEMP.tam.4B.3	BobWhite_c47144_153	50.88	2.58	34.4309	33.8866	9.2	0.27216	TAM 112
STEMP	UD13	QSTEMP.tam.2A.4	RAC875_rep_c69619_145	6.711	2.12	37.6898	36.8193	7.9	0.43526	TAM 112
	UC13	QSTEMP.tam.1B.3	wsnp_Ex_c15611_23928961	115.06	2.05	29.7636	29.1915	7.3	0.28605	TAM 112
	UC13	QSTEMP.tam.2A.4	Tdurum_contig45196_487	96.6	2.42	29.6238	28.9047	8.6	0.35958	TAM 112
	UC13	QSTEMP.tam.3A.1	wt7217	6.197	2.53	29.0504	29.7529	9	-0.3513	TAM 111
	UC13	QSTEMP.tam.3A.1	GBS52472	27.01	2.29	29.0911	29.7028	8.2	-0.3058	TAM 111
SHW	UC13	QSHW.tam.1A.1	RAC875_c43643_176	18.522	2.8	0.78997	0.85854	9.9	-0.0343	TAM 111
	UC13	QSHW.tam.1B.3	BobWhite_c5655_300	142.195	2.99	0.8675	0.79768	10.5	0.03491	TAM 112
	UC13	QSHW.tam.6B.1	GBS11093	134.189	2.68	0.79082	0.85893	9.5	-0.0341	TAM 111
	ET13	QSHW.tam.7D.1	GbDATA2011	235.493	3.75	0.81605	0.74896	13	0.03354	TAM 112
	CS13	QSHW.tam.7A.4	GBS6103	21.039	2.6	1.147	1.23914	9.2	-0.0461	TAM 111
	CS13	QSHW.tam.7B.1a	BobWhite_c44404_312	83.692	3.27	1.1369	1.23912	11.4	-0.0511	TAM 111
	CH13	QSHW.tam.1B.3	Kukri_c5336_365	122.033	3.87	0.99759	0.92166	13.4	0.03797	TAM 112
	CH13	QSHW.tam.7A.4	Tdurum_contig75584_1118	18.741	3.62	0.92449	0.99792	12.6	-0.0367	TAM 111
	UC12	QSHW.tam.6B.1	Excalibur_c36944_509	80.485	2.8	0.87971	0.97635	9.9	-0.0483	TAM 111
	CS12	QSHW.tam.6A.1	GBS66671	4.214	3.79	0.76641	0.86761	13.1	-0.0506	TAM 111
	BD12	QSHW.tam.2A.3	BS00110524_51	3.142	3.01	0.24261	0.27796	10.6	-0.0177	TAM 111
	BD12	QSHW.tam.7D.1	RPM1PIF4R3	248.836	2.68	0.26293	0.23588	9.5	0.01352	TAM 112
KW	UC13	QKW.tam.4B.3	Ku_c103450_879	20.049	2.49	0.0297	0.03102	8.8	-0.0007	TAM 111
	UC13	QKW.tam.2D.1	BobWhite_c9564_587	22.822	2.46	0.03097	0.02965	8.7	0.00066	TAM 112
	UC13	QKW.tam.6A.1	BS00058288_51	6.712	2.98	0.02978	0.03125	10.5	-0.0007	TAM 111
	UC13	QKW.tam.6A.4	Excalibur_c20255_463	40.835	2.66	0.02967	0.03104	9.4	-0.0007	TAM 111
	CS13	QKW.tam.2D.1	GBS82073	63.43	3.62	0.03159	0.02999	12.6	0.0008	TAM 112
	CS13	QKW.tam.7D.1	wsnp_CAP8_rep_c9647_419859	40.534	2.63	0.03158	0.03024	9.3	0.00067	TAM 112
	CH13	QKW.tam.2D.1	wt731062	32.592	4.82	0.03148	0.02943	16.4	0.00102	TAM 112
	CH13	QKW.tam.2D.1	GBS62176	65.947	5.36	0.03142	0.02926	18	0.00108	TAM 112
	CH13	QKW.tam.7D.1	wsnp_CAP8_rep_c9647_419859	40.534	3.59	0.03134	0.0296	12.5	0.00087	TAM 112
	UC12	QKW.tam.7D.1	wt669665	108.064	2.89	0.04253	0.04092	10.2	0.00081	TAM 112
	CS12	QKW.tam.3A.2	Kukri_c93012_76	7.026	2.61	0.04039	0.03842	9.2	0.00098	TAM 112
	CS12	QKW.tam.4B.3	wt5996	44.68	3.09	0.04043	0.03827	10.8	0.00108	TAM 112
	CS12	QKW.tam.7B.1a	BobWhite_c41356_62	94.41	2.91	0.03808	0.04023	10.3	-0.0011	TAM 111
	CS12	QKW.tam.7D.1	GBS56163	210.32	2.68	0.04047	0.03832	9.5	0.00108	TAM 112
	BD12	QKW.tam.5B.1	wt1482	11.413	3.49	0.02229	0.02086	12.2	0.00072	TAM 112

Table 7 Continued

Traits	Location	QTL	Marker	Position	LOD	mu_A	mu_B	% Expl.	Additive	Favorable allele
KNO	UC13	QKNO.tam.1B.3	BobWhite_c5655_300	142.195	5.55	29.3448	26.303	18.6	1.5209	TAM 112
	UC13	QKNO.tam.4A.1	Excalibur_c82040_91	33.726	2.86	28.7727	26.5345	10.1	1.11912	TAM 112
	UC13	QKNO.tam.4A.1	GBS64279	74.754	2.92	28.8791	26.4538	10.3	1.21262	TAM 112
	UC13	QKNO.tam.6B.1	GBS11093	134.189	3.44	26.2694	28.7794	12	-1.255	TAM 111
	ET13	QKNO.tam.6B.1	BS00073879_51	111.177	3.35	30.0134	32.6245	11.7	-1.3055	TAM 111
	ET13	QKNO.tam.7A.4	Tdurum_contig75584_1118	18.741	2.46	30.5846	32.7627	8.7	-1.0891	TAM 111
	ET13	QKNO.tam.7D.1	GbDATA2011	235.493	2.63	32.7901	30.5208	9.3	1.13465	TAM 112
	CS13	QKNO.tam.4A.1	GBS64279	74.754	3.93	40.5394	37.0139	13.6	1.76277	TAM 112
	CS13	QKNO.tam.6B.1	Kukri_c80683_206	125.031	2.95	37.0804	40.0206	10.4	-1.4701	TAM 111
	CS13	QKNO.tam.6B.1	IACX4889	133.035	3.21	37.1667	40.1714	11.2	-1.5024	TAM 111
	CS13	QKNO.tam.7A.4	GBS35165	11.307	3.08	37.4293	40.3723	10.8	-1.4715	TAM 111
	CH13	QKNO.tam.1A.1	Excalibur_c90962_511	66.561	4.15	30.6089	33.2114	14.3	-1.3013	TAM 111
	CH13	QKNO.tam.7A.4	Tdurum_contig75584_1118	18.741	3.77	30.7385	33.2203	13.1	-1.2409	TAM 111
	CH13	QKNO.tam.7B.1a	RFL_Contig124_558	48.727	2.8	33.0508	30.8923	9.9	1.07927	TAM 112
	CH13	QKNO.tam.7D.1	GBS31952	5.14	2.49	30.9324	33.0539	8.8	-1.0608	TAM 111
	CH13	QKNO.tam.7D.2	wt744521	20.772	2.9	30.8246	33.0944	10.2	-1.1349	TAM 111
	CS12	QKNO.tam.1B.3	RAC875_c46093_492	140.922	2.7	21.7797	19.6923	9.5	1.04368	TAM 112
	CS12	QKNO.tam.4B.3	wt4962	69.962	2.9	19.6041	21.8241	10.2	-1.11	TAM 111
	CS12	QKNO.tam.4D	GBS77023	26.893	2.57	21.8267	19.7624	9.1	1.03215	TAM 112
Spike/m2	UC13	QSPM.tam.2AB	Excalibur_c20098_350	140.606	2.45	279.158	307.137	8.7	-13.989	TAM 111
	ET13	QSPM.tam.6B.1	GBS50834	117.643	2.92	269.457	246.556	10.3	11.4506	TAM 112
	CS13	QSPM.tam.7D.1	IACX7721	274.708	2.51	265.052	292.961	8.9	-13.955	TAM 111
	UD12	QSPM.tam.5A.2	Kukri_c37802_1215	76.538	3.55	302.268	348.418	12.3	-23.075	TAM 111
	UC12	QSPM.tam.6B.1	Excalibur_c36944_509	80.485	2.4	295.271	253.151	8.6	21.0603	TAM 112
	BD12	QSPM.tam.4A.1	GBS64279	74.754	2.93	607.579	676.885	10.3	-34.653	TAM 111
	BD12	QSPM.tam.6B.1	GBS33706	207.434	4.93	687.891	604.298	16.7	41.7965	TAM 112
	BD12	QSPM.tam.7A.4	wt9914	6.732	2.74	673.099	608.129	9.7	32.4848	TAM 112
Yield	UC13	QYD.tam.7B.1a	GBS87209	74.354	2.72	2.26899	2.55223	9.6	-0.1416	TAM 111
	ET13	QYD.tam.1B.1	wt2751	4.88	2.8	2.27866	2.41918	9.9	-0.0703	TAM 111
	ET13	QYD.tam.7D.1	wsnp_CAP8_rep_c9647_419859	40.534	2.56	2.28242	2.41536	9.1	-0.0665	TAM 111
	CS13	QYD.tam.1A.2	GBS63811	5.5	2.45	3.13231	3.41979	8.7	-0.1437	TAM 111
	CS13	QYD.tam.2D.1	Ex_c63594_515	41.079	2.48	3.13017	3.41831	8.8	-0.1441	TAM 111
	UD12	QYD.tam.3B.1	wt10948	22.073	3.55	2.9113	3.27101	12.3	-0.1799	TAM 111
	UD12	QYD.tam.4B.3	GBS77499	47.631	2.48	3.22431	2.92166	8.8	0.15133	TAM 112
	UD12	QYD.tam.7B.1a	GBS87209	74.354	2.85	2.91836	3.24175	10.1	-0.1617	TAM 111
	UC12	QYD.tam.3B.1	Tdurum_contig56933_86	15.034	2.44	2.3258	2.61287	8.7	-0.1435	TAM 111
	UC12	QYD.tam.5B.4	wt6348	52.822	2.48	2.3208	2.61032	8.9	-0.1448	TAM 111







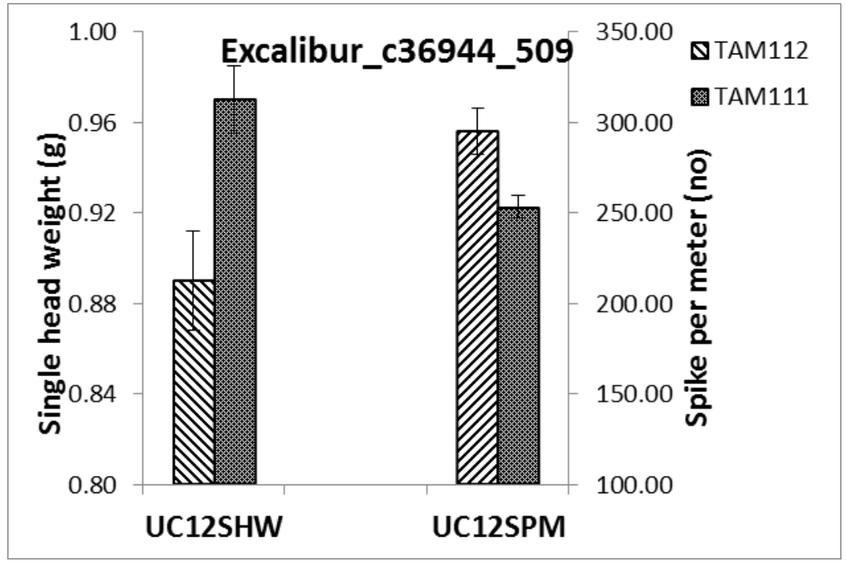
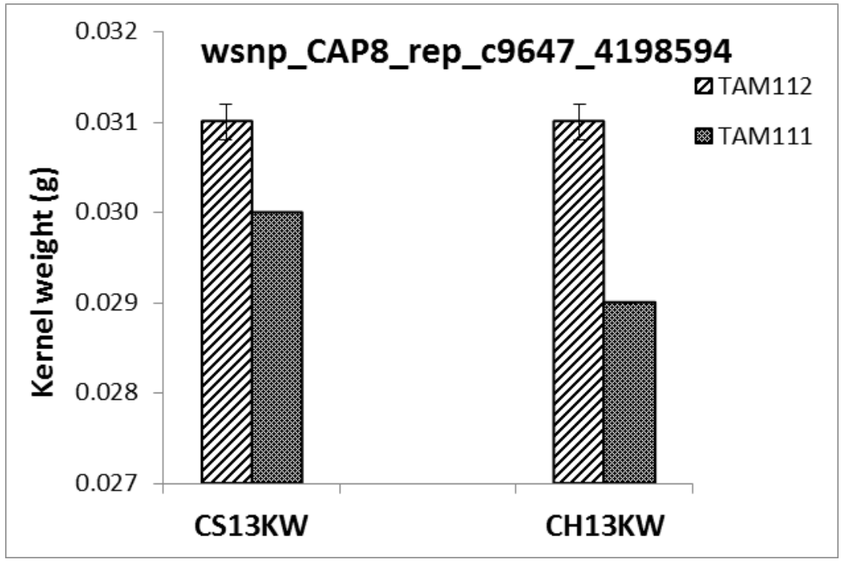
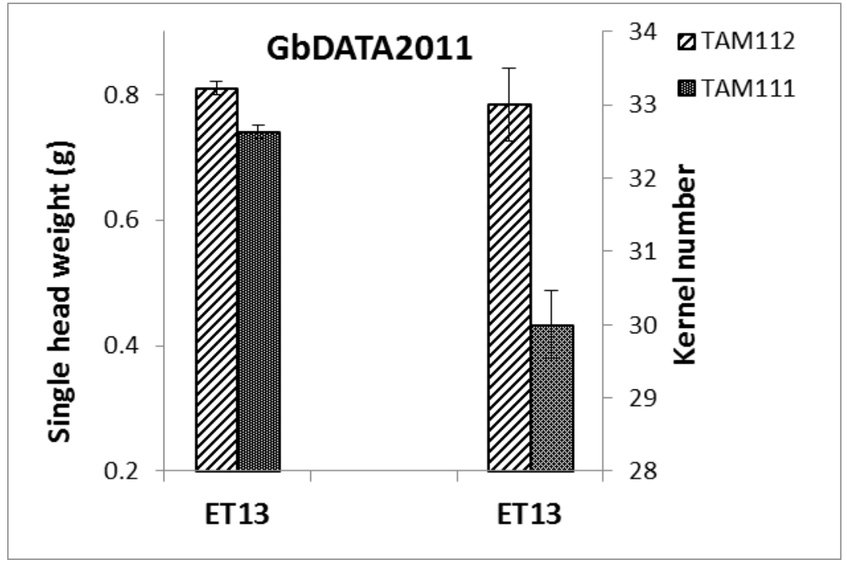


Fig.4 Mean allele values for wax, canopy temperature, yield and yield components having either TAM 112 or TAM 111 allele for different markers across environments





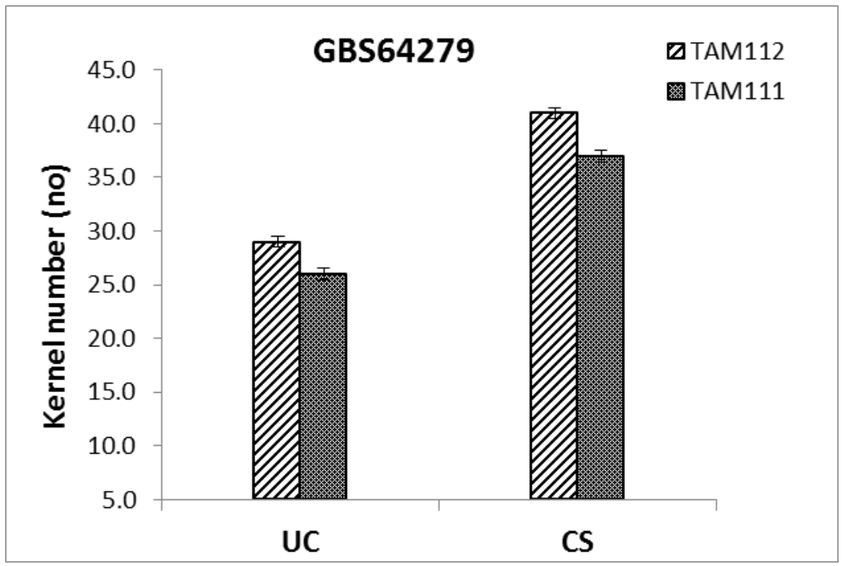


Fig.4 Continued

TAM 111 alleles for yield were associated with chromosomes 1A, 1B, 2D, 3B, 5B, 7B and 7D and 7B, while TAM 112 had favorable alleles on 4B and 7D (Table 7). Both TAM 112 and TAM 111 alleles were also associated with increased spike/m^2 on across various chromosomes. For SHW, again TAM 111 contributed most of the alleles. However, for KWT, TAM 112 contributed the favorable alleles. QTL for leaf and spike temperature was detected in chromosome 1B, 2A, 3A, 3B, 4B and 7D and TAM 112 contributed the favorable alleles.

3.4 Discussion

The 124 RIL populations derived from TAM 111 x TAM 112 showed significant genotypic variation for all the traits. The effect of environment can be inferred from highly significant G x E interaction especially yield, spike/m^2 and leaf wax. The large effect of environment on yield is well known for wheat and barley (Reynolds et al. 1994). However, the performance of the parental cultivars TAM 112 and TAM 111 were not significant for all the traits. Leaf wax is an adaptive trait and environment has significant effect on wax profile (Jenks et al. 1998). The amount of leaf wax in RILs under limited and well irrigated experiments ranged between 1 to 35.29 mg/dm^2 and 1.62 to 36.28 mg/dm^2 respectively, explaining that differences exist within and between plants for leaf wax. Similar response was observed for glume wax. The amount of wax is similar in both control and water deficit treatments. Though, increase in wax deposition has been shown to be a response to water stress, this was not

observed in this study which in agreement with the previous findings (Tischler et al. 1995). The lower range of yield (0.81t/ha) and yield components (0.26, 12g MSH and TKWT respectively) of RILs were lower than the parental lines. The upper range also exceeded both parents suggesting transgressive segregation among the RILs. A similar pattern was observed for all traits in both the treatments.

Leaf wax had a negative correlation with leaf and spike temperature in both the treatments, suggesting that the epicuticular wax significantly reduces canopy temperature and canopy cooling may be plants adaptive mechanism under stress. However, in both the treatments, glume wax did not show an association with spike temperature. In water deficit treatment, yield and yield components were positively associated with the glume wax suggesting that an increase in wax increases yield. Similar reports were observed for wax and yield in peanut. In contrast, both leaf and glume wax had negative association with yield components in well-irrigated treatment. This results are in association with previous findings that wax contributes to yield based on the growing environment conditions such as temperature, rainfall and relative humidity (Wang et al. 2014).

Leaf temperature was positively correlated with spike temperature and negatively with yield and yield components in both the treatments as expected. However, the rate of yield reduction was more pronounced in water deficit treatment. The positive association of yield with yield components in both the treatments suggests

that the total yield is influenced by yield components. Similar observations were reported in wheat grown at different environments.

Crop yield is determined by complex interaction between the genetic make-up of the plant and the environment in which plants are grown. QTL analysis has been used to dissect the genetic regions associated with traits of agronomic importance. In wheat, QTL associated with various physio-morphological traits were identified in different studies (Bonneau et al. 2013). Though few studies have mapped glaucous QTL on chromosomes 1D, 2A, 2B, 2D, 3A, 4A, 4D, 5A, 5B, 6A, and 7A under water deficit and heat stress conditions (Mason et al. 2011; Bennett et al. 2012; Mason et al. 2013) relatively few have identified QTL for epicuticular wax. In this study, 9 QTL associated with leaf and glume wax was identified on chromosome 1B, 3A, 3D, 6B, 7B and 7D. In wheat, previous study detected leaf wax QTL in chromosome 1B, 3D and 5A of RIL population derived from Halberd x Karl92 (Mondal et al. 2015). Both parents TAM 112 and TAM 111 contributed favorable allele, however TAM 112 contributed 7 of 9 wax QTL. Though the glaucous genes W1 and W2 were located on chromosome 2B and 2D respectively, we did not find any wax QTL in chromosome 2.

A genetic link between leaf and glume wax and canopy temperature was observed in this study, with three loci showing close association between these two traits. The glume wax QTL on 1B was in close association with QTL for spike temperature, whereas the leaf and glume wax QTL on 3A was linked with leaf temperature suggesting that these two traits are influenced by common genetic loci

(Fig.3) and could be useful for improved adaptation under stress conditions. Similar results showing co-localization between wax and canopy temperature was reported in wheat (Mondal et al. 2015). The QTL for CT on chromosome 1B was also reported previously in different study (Rebetzke et al. 2012). The favorable allele for the wax and leaf temperature was contributed by the TAM 112 parent, confirming its adaptation to water deficit conditions.

A number of QTL were identified for yield and yield components including MSHW, TKWT, spike/m^2, grain filling rate and biomass production on chromosomes 1B, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 5A, 5D, 6B, 7B, 7D in different studies (Mason et al. 2013; Talukder et al. 2014). In this study, QTL for yield and yield components were identified in the seven of the following chromosomes: 1B, 2B, 3A, 3B, 3D and 7B, 7D (Fig.3). Among the seven QTL, two QTL each on 1B and 6B was detected for SHW. The QTL for spike/m^2 was co-localized with yield QTL in 2B, 3B, 6D and 7D chromosomal regions. The 2B chromosome for yield have been mapped previously (Bennett et al. 2012). Many of the yield and yield component QTL reported herein were consistent with QTL reported in other studies (Bonneau et al. 2013). On chromosome 7D, leaf wax QTL was co-localized with spike m^{-2} QTL. This region also included QTL for yield and SHW and has been previously reported for yield and yield component QTL (Mason et al. 2013). QTL for yield, spike/ m^2 and leaf temperature were detected on chromosome 3B under drought stress environment. Similar result were reported in a DH population in which the QTL on chromosome 3B was found to be associated with yield, TKWT and canopy

temperature under drought and heat stress conditions (Bonneau et al. 2013). When QTLs for different traits overlapped, the favorable alleles seem to be coming from the same parent for the overlapped traits (Fig.4). In our study, the parent TAM 111 contributed the favorable QTL allele for grain yield and spike m^2 whereas the favorable allele for wax and canopy temperature came from the parent TAM 112.

3.5 Conclusion

The study was aimed at identifying the association between epicuticular wax, canopy temperature and yield in a 124 RIL population derived from a cross between TAM 111 and TAM 112 under water deficit and irrigated conditions across locations for two years. Though in one location glume wax had significant association with yield (CS 2013), in UD 2013 it showed negative correlation. canopy temperature, with the population showing significant variation for these two traits. Further, identification of co-localized QTLs for these traits suggests that they may be genetically linked and can be used to develop improved stress tolerant wheat cultivars. The epicuticular wax had correlation with yield components in both control and water deficit environments, but the association were varied between environments. However, further research is needed to validate the identified QTL to be used in marker-assisted selection.

4. QTL MAPPING FOR QUALITY, YIELD AND YIELD COMPONENTS IN TAM 112 X TAM 111 RECOMBINANT INBRED LINES

4.1 Introduction

Wheat is one of the primary sources of calories for millions of people and the wheat grain is usually converted into various value added products. Each product is based on the milling and baking properties of the grain. As a result, wheat is marketed into distinct classes such as hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), durum, hard white (HW), and soft white (SW) wheat. The HRW wheat is planted in the fall and accounts for more than 40% of the U.S wheat crop. The U.S exports about 50% of its total production and one-third of its HRW wheat. HRW wheat is grown over a wide area of the great plains of the U.S and Texas is one of the major producers of the HRW. The HRW wheat has medium to high protein and excellent milling and baking qualities, which makes it the principle source of flour for bread (USwheat.org). Some of the key traits for a quality bread loaf are: kernel hardness, protein, moisture, and peak time. Kernel hardness is defined as a resistance to cracking, an indicator of milling quality of wheat and is associated with the protein friablin. The protein friablin consists of two proteins, puroindoline a and b (pin a and pin b), with pin a being present in soft wheat and pin b in hard wheat (Giroux et al. 1998; Salmanowicz et al. 2012). Grain hardness is controlled by a single major gene (Ha), located on the short arm of chromosome 5D and it influences grain sifting, flour particle size, water

absorption and fermentation apart from separating the wheat into distinct market classes, hard and soft (Li et al. 2011; Salmanowicz et al. 2012). Grain hardness is also influenced by protein content and a significant positive correlation was reported between hardness and protein (Pasha et al. 2010). The presence of specific proteins and protein subunits play the most decisive factor in bread-making quality. The proteins, glutenin and gliadin are responsible for the extensibility of the dough and strong elastic gluten is considered good for bread-making whereas, low protein content, weak gluten, is used in other baking products. The genetic factors influencing protein is distributed all over the wheat chromosomes and the protein is highly influenced by large environmental factors (Li et al. 2011). The protein content influences water absorption and the flour with high water absorption requiring less flour (Tsilo et al. 2013). The peak time is the time required for the dough to reach its maximum consistency and provides information about the relative strength of the dough (Kharel et al. 2011). It has been well documented that water absorption, dough development time and dough stability are strongly correlated with protein content. Apart from hardness and protein content, test weight is another key trait that determines the market grade of the wheat. Test weight is a measure of the density of the grain and an overall indicator of grain quality, which gives an estimation of potential flour yield. Larger kernels yields more flour and get a high market price (Ramya et al. 2010). Yet, the quality traits are greatly influenced by genetics (G) and environment (E) and the interaction between GxE. The environmental conditions such

as temperature, drought, precipitation and late season frost affects metabolic processes thereby expression of end-use quality traits thus emphasizing the need to conduct evaluation trials across environments (Peterson et al. 1989; Mut et al. 2010).

4.1.1 Impact of heat and drought on wheat quality

A number of studies have shown the effect of both high temperature and drought stress on grain quality. The percentage of protein increases with rise in temperature however, temperature above 30°C during grain filling reduces the amount of both protein and starch (Bhullar et al. 1985). It was also found that increase in temperature decreases loaf volume and mixing time. High temperature elevates protein concentration but reduces protein functionality. In addition, high temperature affects the rate of carbon and nitrogen deposition in the grain (Al-khatib et al. 1984). The timing and duration of heat stress causes variation in dough properties (Castro et al. 2007). Heat stress affects synthesis, accumulation and assembly of gluten proteins. A short period of temperature above 30°C produces weaker dough due to a change in glutenin:gliadin ratio and reduces the activity of starch synthase (Jenner 1994).

In addition, heat and drought stress, greatly affects gluten macropolymer (GMP), a high molecular weight gluten protein, widely accepted as predictor of end-use quality. Drought stress during grain filling also decreases the dough mixing time and gluten elasticity by weakening the gluten. It was found that water deficit conditions affect high and low molecular weight-glutenin subunits (HMW-GS, LMW-GS) ratio and

gluten strength (Flagella et al. 2010). In addition, it reduces the volume and surface area of B-type starch granules. High temperatures and drought stress occur more frequently in most of the wheat growing regions during grain filling, so there is a need for direct or indirect selection of physio-morphological traits conferring more stable end-use quality under stress conditions. Glaucous or epicuticular wax accumulation is a response to stress conditions and studies showed that glaucous surface reflects excess light and thus reducing canopy temperature (Shepherd et al. 2006). Further, lower rate of water loss observed in glaucous plants is considered as a drought survival mechanism (Clarke et al. 1988). Although the effects of wax on heat and drought tolerance are documented, there is no information on how and to what extent it influences grain quality, if any. To understand the effect of epicuticular wax on quality traits, we investigated the association between some quality and yield parameters in a RIL population grown under different treatments across Texas.

4.1.2 Quality QTLs

Many quality traits are quantitative in nature and thus QTL analysis is an effective way to dissect the loci controlling the traits that will eventually be used in marker assisted selection. Many QTLs for various quality traits have been identified in different populations including RILs, double haploids and other populations. The QTLs identified were distributed across all 21 wheat chromosomes (Sun et al. 2008). Grain hardness QTLs were identified in different chromosomes including 1A, 1B, 2A, 5A, 7A,

7B, 2D, 5D and 6D. Further, the QTLs identified for protein and hardness in 2B and 6B in a inbred line population suggested that loci may be linked to each other (Salmanowicz et al. 2012). Though most of wheat storage proteins were identified on chromosome group of 1A, 1B and 1D, many authors reported protein QTLs across different chromosomes. A study identified 15 QTLs mainly on chromosome 1D, 3B and 6D for protein related traits. However, other authors have identified protein QTLs in 1A, 2B, 5A, 7A, 2D and 5D chromosomes of bread wheat and 4B, 6B, 7B, 5A and 6A chromosome of durum wheat. In different studies, QTL for kernel diameter in 3B and mixograph peak time QTLs on 2B and 7B were reported (Mergoum et al. 2013). In addition, flour yield QTLs were detected in chromosome regions of 1B, 2B, 4B, 5A, 2D, 4D, 5D and 7A (Lehmensiek et al. 2006). In a double haploid population 18 QTLs for flour yield were identified. The objective of this study was to:

(i) identify the relationships between epicuticular wax, a drought resistant trait and quality parameters

(ii) identify the genetic loci to determine which parents contribute to favorable alleles for specific QTL for a given trait.

4.2 Materials and methods

4.2.1 Plant materials

The study was conducted in a set of 124 recombinant inbred lines (RILs) derived from a cross between hard red winter TAM 112 and TAM 111. Both parents and the

RILs were grown under different environments in Texas in two growing seasons. Initial screening of quality traits was conducted in the parents TAM 112 and TAM 111 to minimize the time and resources. Samples from seven locations were tested for ten quality traits and based on the results and the availability of seeds, Bushland (BD), Chillicothe (CH) and Etter (EP-65), TX locations had been chosen for further analysis in the year 2012 (data not shown). In ET, the experiments were laid out on 40, 50, 65, 75 and 100 evapotranspiration levels (EP) and for this experiment EP-65 was used. In 2013, the samples collected from College Station (CS) were used. BD and CH was water deficit environments whereas at the CS trial was an irrigated environment. The lines were tested for quality parameters such as kernel hardness, diameter and kernel weight using Single Kernel Characterization Systems (SKCS). Protein and moisture content were determined using Near Infrared Reflectance Spectrometry (NIR); flour weight and peak time were obtained from mixograph results.

Though the parents and RILs were evaluated in randomized complete block design (RCBD) with two replications in the year 2012 and 2013, only one rep was used for this experiment except yield and yield components. The SKCS (SKCS4100, Perten Instruments North America Inc., Spring-field, IL, USA) uses 300 kernels to measure hardness, weight, diameter and moisture. The kernel hardness is dimensionless and is expressed from 20 to 120 with 20 being soft to 120 being hard. About 130 grams of samples were tempered overnight to 14% moisture content and then milled using Brabender Quadromat Mill (C.W. Brabender Instruments, South Hackensack, NJ). The

flour weight separated from the bran and the weight was calculated using a weighing machine. Flour protein content was determined by NIR. For mixograph (National Mfg Co., Lincoln, NE, USA) analysis 10 grams of flour was used. The mixograph peak time was measured and the data was collected using Mixsmart software program.

4.2.2 Statistical analysis

The phenotypic data collected across environments were analyzed using SAS 9.2. LS means was used to differentiate the parents for all the traits across environments. The frequency distribution was analyzed by PROC UNIVARIATE procedure. Phenotypic correlation among the traits was estimated using PROC CORR procedure. Wax samples were collected from BD and CS locations but not in CH and ET-65 in 2012. Thus for correlation analysis, the samples from BD and CS were used.

4.2.3 QTL analysis

DNA was extracted from the leaf tissue of both parents and RILs and was genotyped at the USDA- ARS, Fargo, North Dakota. High throughput genotyping of 90,000 (90K) SNP (single nucleotide polymorphism) was performed using Illumina's golden gate assay. The 90K SNP clustering and annotation was performed using genome studio software. The markers identified as polymorphic (3166 marker) were used to construct linkage group using JoinMap 4 software with regression mapping method and Kosambi mapping function. The resultant linkage groups were used to detect QTL using MapQTL 6 software. Multiple QTL Mapping (MQM) analysis was

conducted across environments to detect main effect QTLs. For linkage group construction a significance level of 0.05 was set and for QTL mapping 10000 permutations were used to determine the maximum likelihood of odds (LOD) score threshold. A QTL was determined to be present if the LOD score value is 2.5 and above and the QTL was considered to be stable if it present in at least two environments. Graphical representation of QTLs was performed using MapChart 2.2 software.

4.3 Results

Table 8 shows the seasonal variation during the growing season, monthly maximum and minimum temperature and average precipitation at each of the locations. Bushland is a dry environment so as Chillicothe with hot summer and cold winter months. Chillicothe had maximum temperature during the growing season compared to other environments. College Station received the maximum precipitation apart from the supplemental irrigation.

Table 8 Monthly minimum and maximum temperatures and average precipitation across four locations during the years 2012 and 2013

Year	Locations	Temperature (°F)						Precipitation (inch)		
		Mar		Apr		May		Mar	Apr	May
		Min	Max	Min	Max	Min	Max			
2012	Bushland (BD)	41	72	48	78	55	84	0.04	0.07	0.05
	Etter (ET)	39	70	45	75	53	82	0.03	0.07	0.00
	Chillicothe (CH)	49	75	56	84	65	103	0.05	0.05	0.02
2013	College station (CS)	48	73	56	77	64	85	0.06	0.07	0.25

The grain yield between the parental lines was significant in CH among the four locations whereas test weight significantly differed between the parents in both CH and BD (data not shown). The kernel hardness of TAM 111 and 112 was significant in all locations except Etter which could be due to the influence of environmental factors. The protein content between the two parental varieties didn't show any significance across locations so as other traits such as kernel weight, diameter, flour weight, water absorption and peak time (data not shown). However, the range values between the parents and RILs showed (Table 9) variation for all quality and yield traits measured. Though the lower range of the RILs were lower than the two parental varieties the upper range exceeded both parents in all the locations and varied depending on environment. For example, in Bushland, hardness value for TAM 111 ranged from 57.4 – 65.0 and TAM 112 ranged from 70.0 – 74.1 whereas, the RIL showed variation from 50.4 – 81.4. The upper range was higher than both parents. Similarly, for peak time in CH, the TAM 111 ranged from 3.3 – 5.0 min and TAM 112 ranged from 4.3 – 5.3 min whereas, the RIL showed ranged from 1.5 – 7.3 min. Similar pattern of results were observed for all the other traits indicating evidence for transgressive segregation in the population (Table 9 and Fig.5).

Table 9 Range values for agronomic and SKCS quality traits for the RILs and parents across locations during the years 2012 and 2013

Locations	Grain yield (tons/ha)	Test weight (g)	Hardness	Weight (mg)	Diameter (mm)
BD-RILs	0.81 – 2.17	53.3 - 60.6	50.4 - 81.4	22.3 - 29.2	2.3 - 2.6
TAM 111	1.26 – 1.67	56.9 - 59.2	57.4 - 65.0	24.6 - 26.8	2.4 - 2.5
TAM 112	1.29 – 1.90	56.1 - 58.0	70.0 - 74.1	23.5 - 26.7	2.3 - 2.4
CH-RILs	1.54 – 3.17	53.5 - 62.6	54.0 - 87.7	22.0 - 29.1	2.3 - 2.6
TAM 111	1.95 – 2.74	57.2 - 59.2	62.2 - 65.0	25.5 - 25.9	2.4 - 2.5
TAM 112	2.44 – 2.95	59.2 - 60.6	82.6 - 85.9	24.9 - 25.3	2.4 - 2.4
ET65-RILs	0.73 – 2.39	49.5 - 57.0	49.4 - 74.3	21.2 - 28.9	2.3 - 2.6
TAM 111	1.40 – 1.49	54.0 - 55.5	49.8 - 70.0	21.0 - 27.8	2.3 - 2.5
TAM 112	1.20 – 1.65	54.0 - 55.0	54.2 - 59.0	24.6 - 28.7	2.4 - 2.6
CS-RILs	1.14 – 4.62	52.9 - 62.0	48.7 - 82.5	22.3 - 35.5	2.3 - 2.8
TAM 111	3.11 – 4.40	58.2 - 60.0	63.6 - 70.9	27.1 - 31.2	2.5 - 2.7
TAM 112	3.33 – 4.62	55.9 - 58.5	59.4 - 69.8	26.3 - 31.1	2.5 - 2.6

Table 9 Continued

Locations	Flour weight (g)	Moisture (%)	Protein (%)	Peak time (min)
BD-RILs	51.6 - 62.9	13.1 -13.8	9.67 -14.7	1.4 - 7.1
TAM 111	56.8 - 59.4	13.3 -13.4	11.2 -14.0	2.2 - 3.0
TAM 112	53.8 - 57.3	13.3 -13.5	11.1 -14.3	2.4 - 4.2
CH-RILs	54.3 - 62.3	13.2 -13.5	11.7 -13.9	1.5 - 7.3
TAM 111	58.2 - 60.8	13.3 - 13.3	12.5 -13.0	3.3 - 5.0
TAM 112	58.0 - 60.2	13.4 - 13.4	12.3 - 12.7	4.3 - 5.3
ET65-RILs	50.5 - 60.4	13.1 - 13.7	13.7 -15.4	2.0 - 7.4
TAM 111	52.0 - 58.6	13.2 - 13.5	14.2 -14.8	2.1 - 5.3
TAM 112	56.8 - 58.4	13.2 - 13.5	13.8 -14.9	2.2 - 6.4
CS-RILs	36.4 - 76.0	11.3 - 14.0	8.5 - 12.9	3.0 - 7.8
TAM 111	55.5 - 58.7	11.5 - 13.7	9.5 - 12.3	1.8 - 4.7
TAM 112	55.6 - 59.7	11.7 - 13.5	9.1 - 10.7	4.8 - 6.5

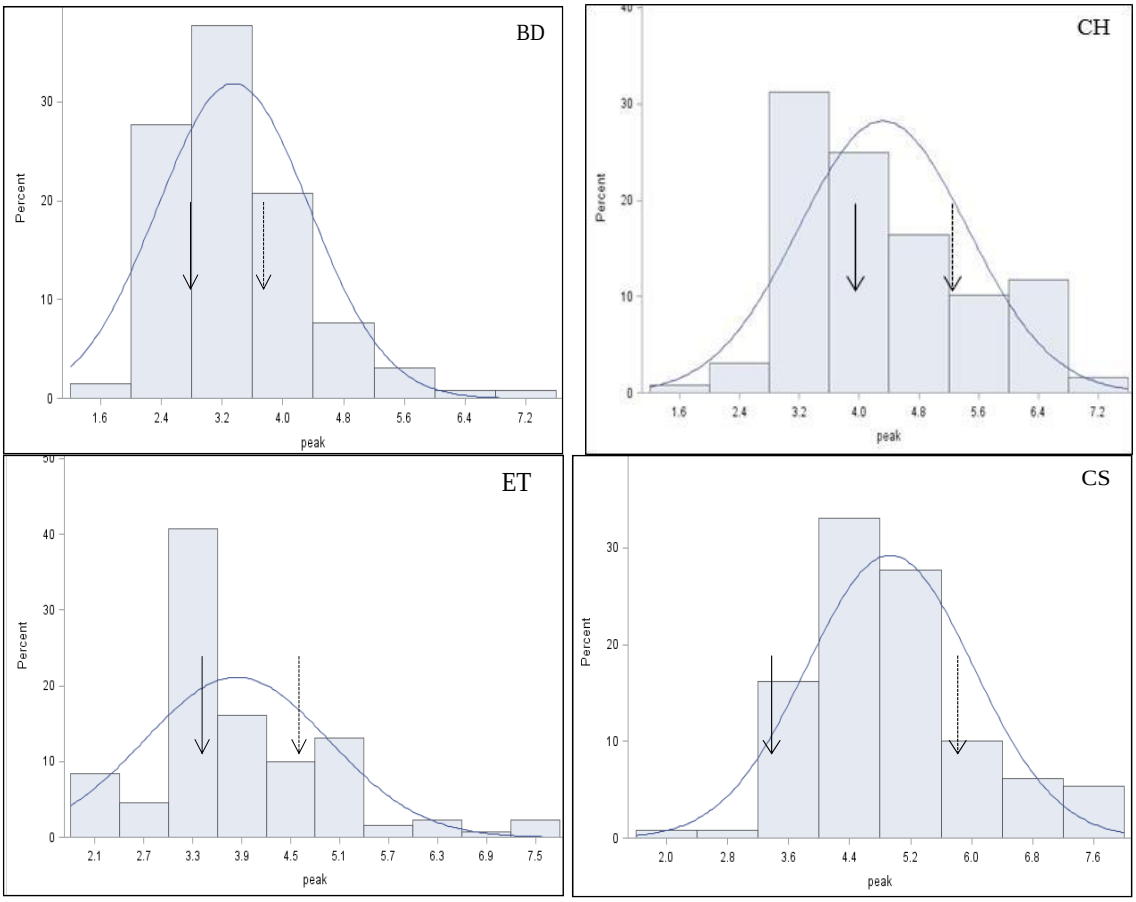


Fig.5 Frequency distribution of mixograph peak time for the RIL populations at different locations in years 2012 and 2013 (Parents are indicated with the arrow → TAM 112; ⇢ TAM111

The mean, coefficient of variation (CV) and mean squares of the combined analysis of RILs are shown in the Table 10. The RILs were significant for all the traits except moisture, protein and leaf wax in the combined analysis. Mixograph peak time showed an average of 4.10 minutes as optimum dough development time for the dough to reach its maximum consistency. The higher CV (40 and 22 percent) for wax

traits showed that they are highly influenced by environment. The protein content varied from 8.5 to 15.4 percent, with an average of 12.8 percent.

Table 10 Mean squares, co-efficient of variation (CV) and mean values of RILs for the combined analysis across environments in 2012-13

Traits	Mean Squares	CV	Mean
Hardness	85.31***	8.06	65.93
ł Weight (mg)	6.09***	6.58	25.91
Diameter (mm)	0.01***	2.8	2.46
Moisture (%)	0.17^{NS}	3.31	13.3
Flour weight (g)	6.81**	3.67	57.47
Protein (%)	0.53^{NS}	6.03	12.81
Yield (t/ha)	0.17*	17.07	2.17
Test weight (g)	13.50***	5.06	56.71
Kernel weight (g)	0.00***	6.23	0.02
Kernel number (no)	14.61*	13.18	25.28
Spike/m^2 (no)	7132.16*	15.71	459.77
Peak time (min)	1.96***	23.03	4.1

***, **, * significant at probability level p ≥ 0.001, 0.01, 0.05 respectively. ł Kernel weight from SKCS

The parental cultivars used in this experiment were selected for a variety of agronomic and end-use quality traits and alleles from both parents contributed to the variation in the RIL. The relation between yield and SKCS quality traits were positive except moisture (r-0.29) and protein (r= -0.80), which showed negative association, as expected. A similar trend was observed for test weight and other quality traits. Leaf wax was negatively associated with yield, TW, peak time and SKCS parameters but showed positive association with moisture and protein. Interestingly, glume wax had quite opposite results compared to leaf wax. Glume wax showed positive association with yield, TW and SKCS traits but negative correlation with protein and moisture (Table 11) suggesting need for further research to identify the interaction between leaf and glume wax and its association with quality and yield traits.

Yield and SKCS parameters including kernel weight (SKW) and diameter (SDIA) showed positive association with yield (r= 0.63 and 0.78). Kernel hardness, weight and diameter showed negative association with moisture and protein but positive association with peak time. Kernel hardness was negatively correlated with SKCS kernel weight. Kernel weight was highly correlated with kernel diameter. Moisture content showed positive relation with protein. Peak time was negatively associated with protein (r= -0.57) and but positively correlated with yield, and SKCS parameters, kernel hardness, weight and diameter (Table 11).

Table 11 Correlations between epicuticular wax and quality traits of RILs in BD and CS environments

Traits	TW	SKW	SDIA	Moisture	Protein	PT	Lfwax	Gluwax	KW	KNO
Yield	0.48***	0.63***	0.78***	- 0.29***	- 0.80***	0.58***	- 0.38***	0.73***	0.85***	0.88***
TW		0.59***	0.64***	-	-0.38***	0.30***	-0.18**	0.33***	0.50***	0.37***
SHD		-0.20**	-	-0.12*	-	0.12**	- 0.21***	0.13*	-	0.16**
SKW			0.89***	-0.17***	-0.53***	0.36***	- 0.16**	0.48***	0.71***	0.58***
SDIA				-0.22***	-0.69***	0.47***	-0.26***	0.58***	0.81***	0.72***
Moisture			0.26***	-		0.12*	-0.36***	-0.31***		-0.32***
Protein				-0.57***		0.41***	-0.59***	-0.75***		-0.75***
PT						-0.32***	0.40***	0.54***		0.57***
Lfwax							-0.26***	-0.40***		-0.41***
Gluwax								0.73***		0.75***
KW										0.86***

***, **, * significant at probability level p ≥ 0.001, 0.01 and 0.05 respectively TW – Test weight, KH- kernel hardness, KW – kernel weight, KD – kernel diameter, WA – water absorption, PT – peak time; S-SKCS values

QTL mapping was performed in the RILs for quality and yield traits using MapQTL and MQM with 10000 permutations for each trait. In total, 71 QTLs for the different traits were detected across environments. Hardness and SKCS kernel weight QTL were identified in three among four locations whereas kernel diameter QTL was identified in two locations (CH and CS). QTL for peak time was identified at 1D2 chromosome for three locations at the same region along with 1 QTL each in 1B and 7B chromosome. Though the QTLs were detected in all three A, B and D genome, the chromosome region of 7D followed by 1D had the most of the QTLs for traits including protein, peak time, hardness, kernel diameter, kernel weight, test weight, yield and wax (Fig.6).

A total of 14 QTL for hardness were identified in chromosome 1A1, 1A2, 1B1, 1B3, 1D2, 2BL, 7D1 and 7D2. The QTLs for hardness from three environments were found to be co-localized in 1B3 region. QTLs that are detected in at least two environments are considered to be stable. A number of putative QTLs were identified in this study for various traits. Three HD-QTL were detected in chromosome 1D2 along with one QTL for SKCS diameter. In chromosome 1B1, QTL for moisture, peak time and hardness were found to be co-localized (Fig.6). In chromosome 2D1, QTL for SKCS kernel weight and diameter were found to be co-localized. In chromosome 4D, 2 QTL each for flour weight and test weight have been identified. QTL for yield components such as spike/m^2, test weight and kernel number along with QTL for moisture were identified in 6B region of the chromosome. The 7D1 chromosome had QTL for

hardness, diameter, kernel weight, yield, test weight, spike/m^2 and leaf wax. Among the QTL, the kernel weight and diameter were closely associated with hardness QTL (Fig.6). In this study, five QTLs were mapped around Glu-D1 locus on chromosome 1D, which is consistent with the results from others. The QTL on chromosome 1A (QHD.tam.1A.1, R^2=38.6%) with TAM 112 contributing favorable allele accounted for higher percentage of genetic variation among the HD QTLs (Table 12). The QTL with highest percentage variation for SKCS weight and diameter was located on chromosome 7D and 2D (QSKW.tam.7D.1, R^2=13.8% and QSDIA.tam.2D.1, R^2=16.6%) respectively.

The QTL with largest variation for peak time was located on chromosome 1D (QPKT.tam.1D.2, R^2=41.8%) and for flour weight the QTL was identified on chromosome 4D (QFLW.tam.4D, R^2=12.2%) respectively. The QTL, QPRO.tam.1D (R^2=11%) and QMO.tam.4A.3 (R^2=13.2%) was associated with the highest percentage of genetic variation for protein and moisture and was contributed by TAM 111 and TAM 112 respectively. Among yield components, spike/m^2 QTL, QSPM.tam.6B.1 (R^2=16.7%), kernel weight, QKW.tam.2D.1 (R^2=12.6%) and kernel number QTL, QKNO.tam.6B.1 (R2=11.2%) showed the highest genetic variation.

Furthermore, QTL for HD was co-located with QTL for MO in chromosome 1B1. These two QTL were also seen to be closely associated with QTL for PK. The SKCS kernel diameter QTL located on chromosome 7D was co-localized with a QTL for QKW, while QTL for SKW, SDIA and HD QTL (Fig.6). In addition, QTL from different location for a

same trait was aligned in a chromosome suggests that the QTL have potential value. For example, BD and CH locations had QTL for HD on 1B3, 1D2, 2BL whereas, PKT QTL on 1D2, SKW QTL on 2D1, TW and FLW QTL on 4D. Further, non co-localized QTL for both quality and yield and yield components were identified across A, B and D chromosomes (Fig.6). Further, mean allele contrast analysis for various quality traits including hardness, peak time, kernel weight and diameter were presented in Fig.7. Though both TAM 112 and TAM 111 alleles contributed for all the traits, in this study, TAM 112 allele contributed mainly for hardness (HD), peak time (PKT), moisture and flour weight (FLW) across environments.

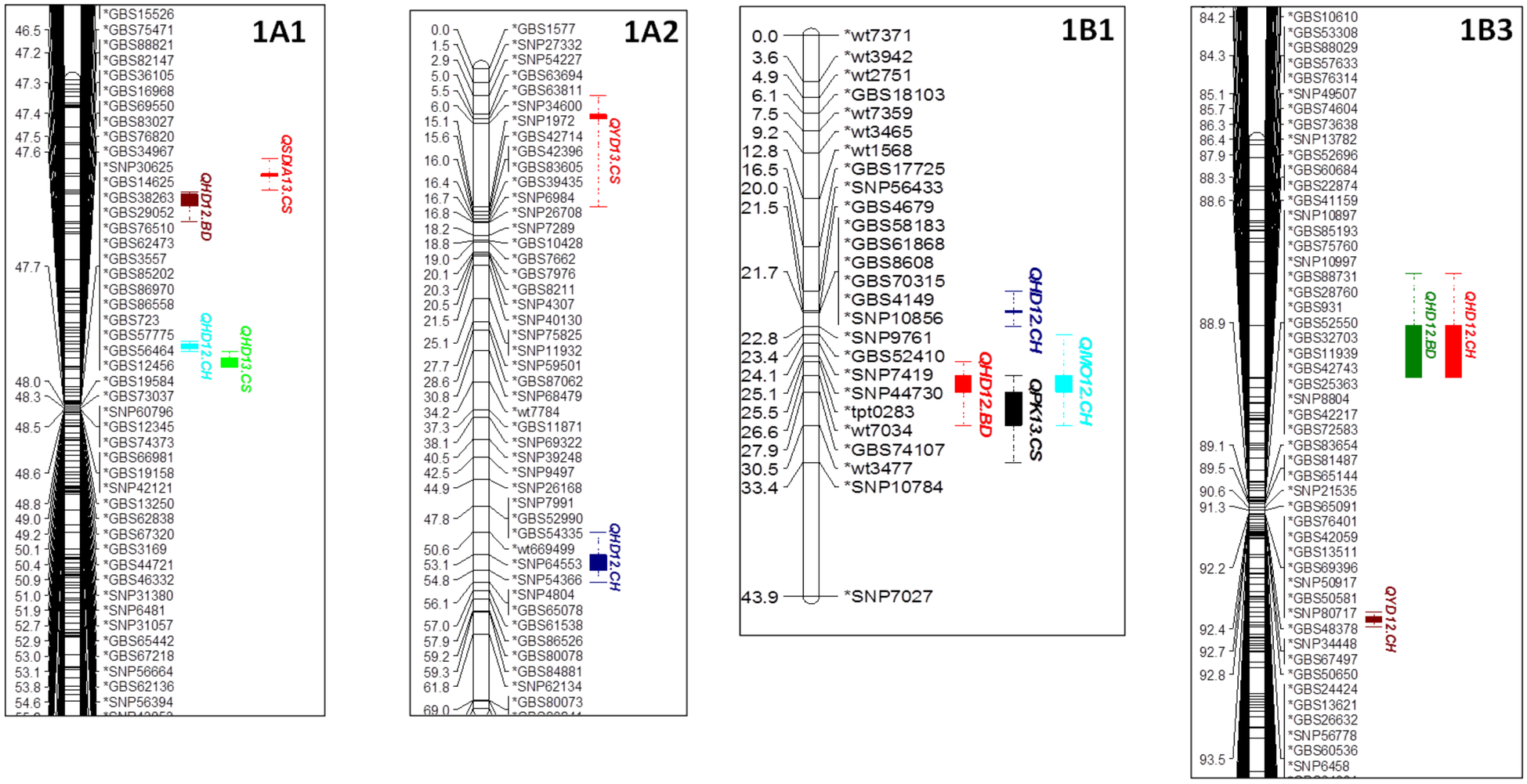


Fig. 6 Linkage map and quantitative trait loci for quality traits, epicuticular wax, yield and yield components in the TAM 111 x TAM 112 RIL population across environments. Marker positions are presented in cM (12, 13 – year 2012 and 2013; CS – College Station; BD – Bushland; CH – Chillicothe)

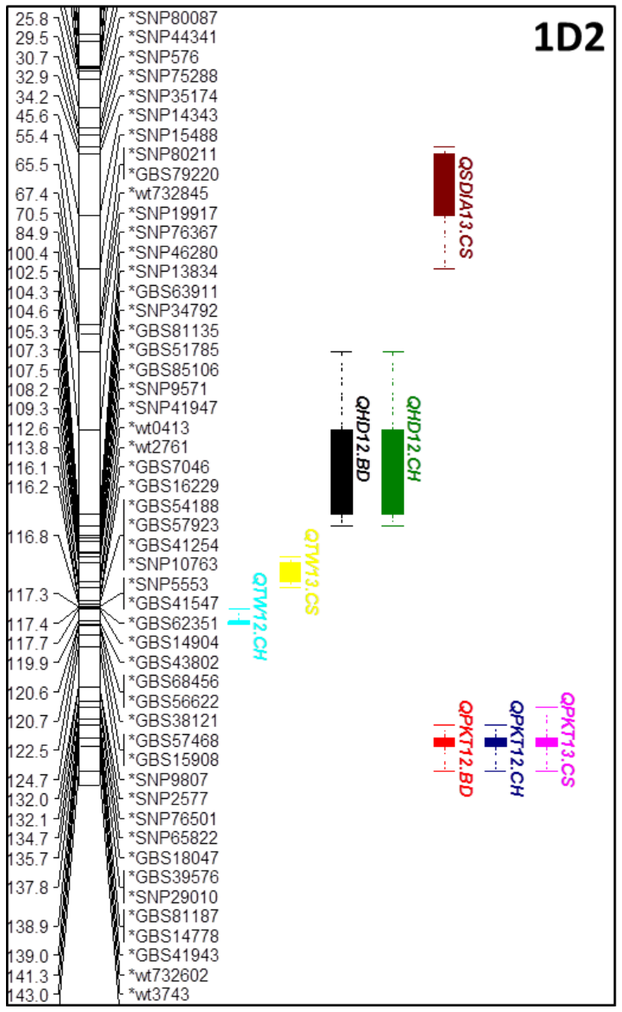


Fig. 6 Continued

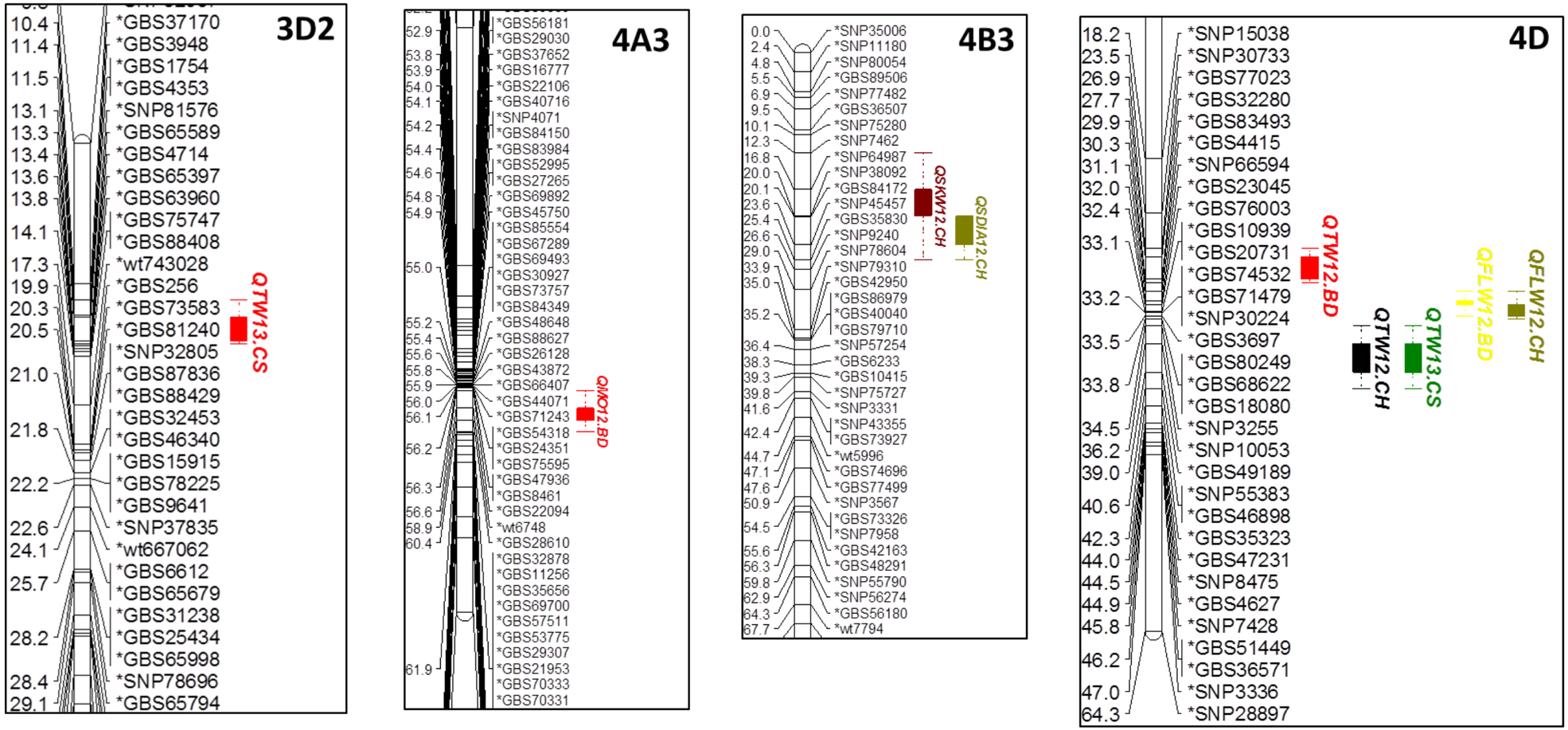
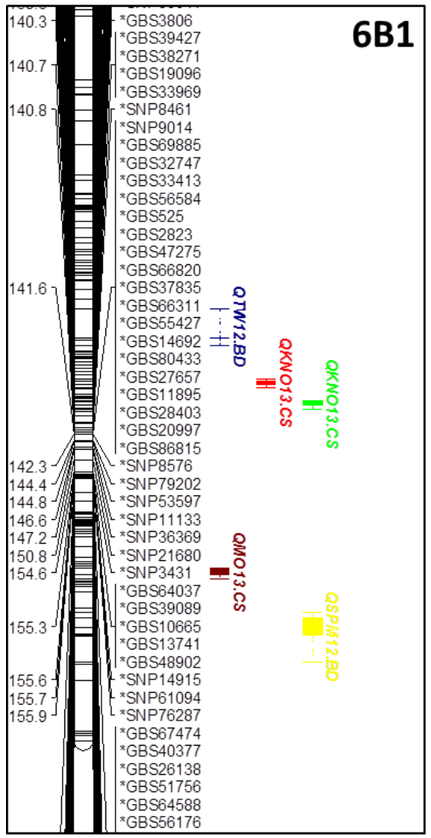
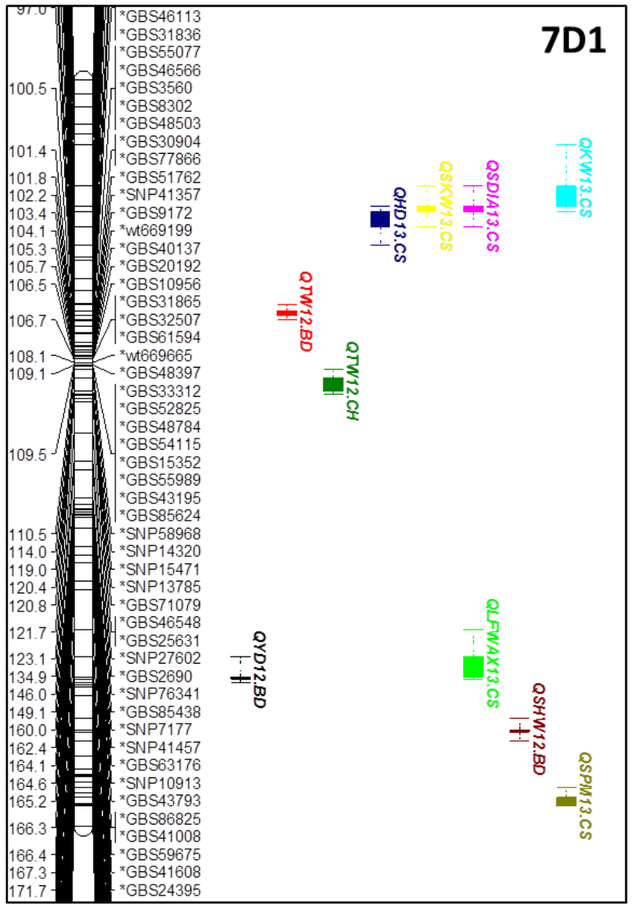
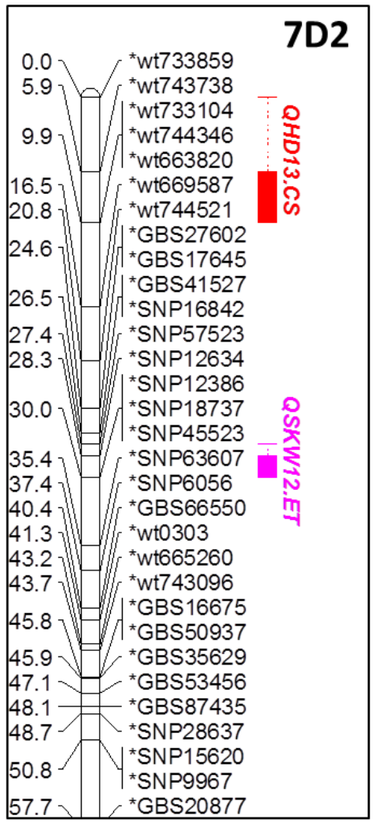


Fig. 6 Continued







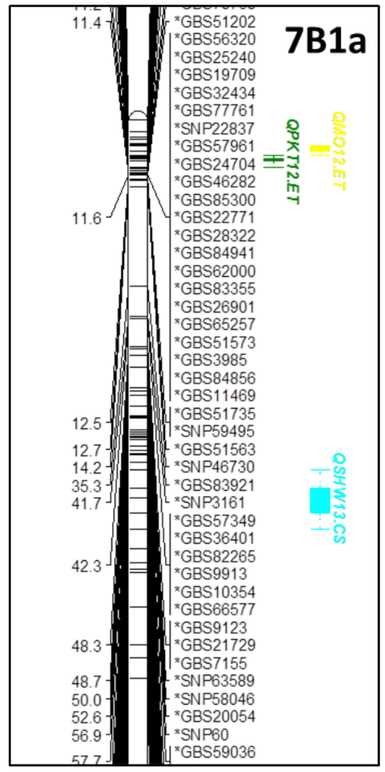


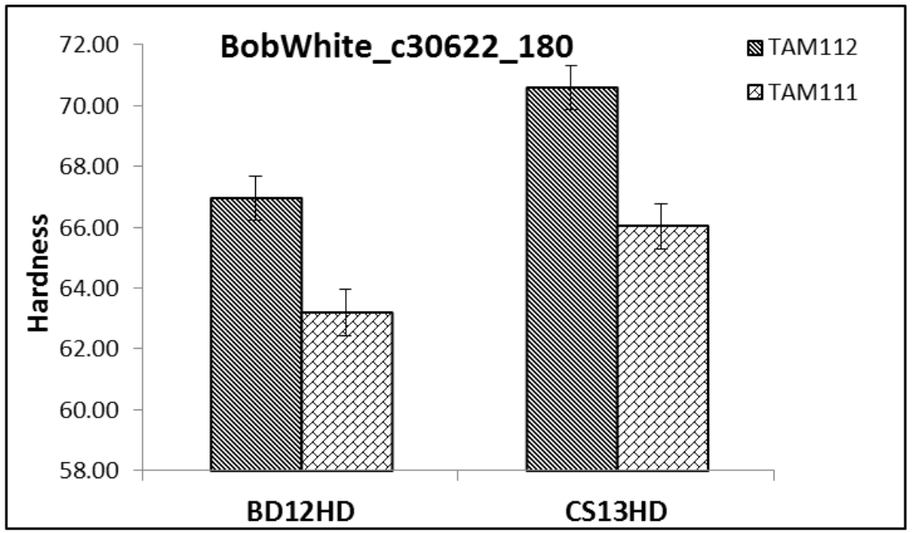
Fig. 6 Continued

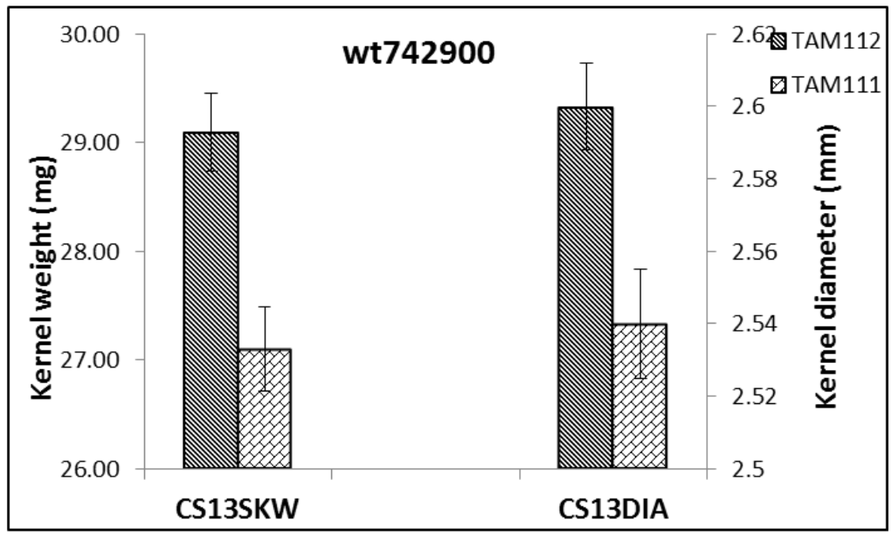
Table 12 Summary of QTL detected in the TAM 112 x TAM 111 populations for quality traits across four environments

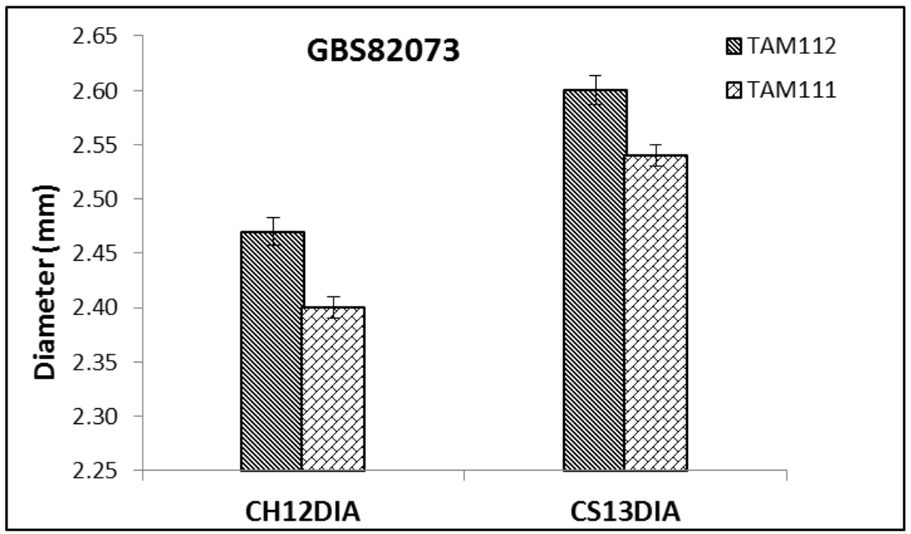
Quality traits	Location	QTL	Marker	Position	LOD	mu_A	mu_H	mu_B	% Expl	Additive	Fav allele
Hardness	BD12	QHD.tam.1A.1	RAC875_c43643_176	18.522	11.58	69.2767	65.5945	61.9124	35	3.68214	TAM 112
	BD12	QHD.tam.1B.1	wt7034	26.623	6.07	67.7706	64.9965	62.2224	20.2	2.77407	TAM 112
	BD12	QHD.tam.1B.3	Kukri_c11587_99	45.722	5.54	66.7006	63.8249	60.9492	18.6	2.87566	TAM 112
	BD12	QHD.tam.1D.2	wsnp_Ex_c12012_19240943	84.899	3.34	66.4059	64.1576	61.9094	11.7	2.24826	TAM 112
	BD12	QHD.tam.2BL	BobWhite_c30622_180	34.183	2.54	66.9661	65.1198	63.2735	9	1.84627	TAM 112
	CH12	QHD.tam.1A.1	wt0515	39.469	13.01	75.9998	71.4768	66.9538	38.6	4.52304	TAM 112
	CH12	QHD.tam.1A.2	RFL_Contig399_1005	53.083	3.14	73.6	71.2008	68.8016	11.1	2.39918	TAM 112
	CH12	QHD.tam.1B.1	GBS58183	21.67	6.5	74.7623	71.4078	68.0533	21.6	3.35451	TAM 112
	CH12	QHD.tam.1B.3	Kukri_c11587_99	45.722	7.71	73.6348	69.7204	65.8059	25.1	3.91445	TAM 112
	CH12	QHD.tam.1D.2	wsnp_Ex_c12012_19240943	84.899	5.61	73.4268	70.0674	66.708	18.9	3.35941	TAM 112
	CS13	QHD.tam.1A.1	wt6654	40.884	4.03	70.7628	68.0371	65.3114	13.9	2.72567	TAM 112
	CS13	QHD.tam.2BL	BobWhite_c30622_180	34.183	3.19	70.6016	68.1693	65.7371	11.2	2.43227	TAM 112
	CS13	QHD.tam.7D.1	GBS46828	56.23	4.47	65.3396	68.3003	71.261	15.3	-2.9607	TAM 111
	CS13	QHD.tam.7D.2	wt743738	5.94	2.85	65.5685	68.1461	70.7238	10.1	-2.5777	TAM 111
SKW	CH12	QSKW.tam.2D.1	wt731062	32.592	3.52	25.5336	24.9684	24.4031	12.4	0.56522	TAM 112
	CH12	QSKW.tam.4B.3	Ku_c103450_879	20.049	2.91	24.4957	24.9942	25.4927	10.3	-0.4985	TAM 111
	ET12	QSKW.tam.7D.2	BS00111202_51	28.271	3.37	24.6524	25.2344	25.8164	11.8	-0.582	TAM 111
	CS13	QSKW.tam.2D.1	wt731062	32.592	2.89	29.0794	28.1772	27.2751	10.2	0.90211	TAM 112
	CS13	QSKW.tam.7D.1	wt742900	48.174	4	29.0571	28.015	26.9728	13.8	1.04215	TAM 112
SDIAMETER	CH12	QSDIA.tam.2D.1	GBS82073	63.43	4.86	2.46932	2.4376	2.40587	16.6	0.03173	TAM 112
	CH12	QSDIA.tam.4B.3	Kukri_c44559_429	23.583	2.8	2.41926	2.44293	2.46659	9.9	-0.0237	TAM 111
	CS13	QSDIA.tam.1A.1	RFL_Contig4231_575	14.226	3.04	2.54211	2.57441	2.60672	10.7	-0.0323	TAM 111
	CS13	QSDIA.tam.1D.2	CAP7_c973_156	45.563	2.97	2.61134	2.57915	2.54695	10.4	0.03219	TAM 112
	CS13	QSDIA.tam.2D.1	GBS82073	63.43	3.02	2.60503	2.57153	2.53804	10.6	0.03349	TAM 112
	CS13	QSDIA.tam.7D.1	wt742900	48.174	3.29	2.60529	2.57082	2.53634	11.5	0.03447	TAM 112

Table 12 Continued

Quality traits	Location	QTL	Marker	Position	LOD	mu_A	mu_H	mu_B	% Exp	Additive	Fav allele
Peaktime	BD12	QPKT.tam.1D.2	wt732602	141.253	14.58	4.02407	3.36481	2.70554	41.8	0.65927	TAM 112
	CH12	QPKT.tam.1D.2	wt732602	141.253	14.58	4.02407	3.36481	2.70554	41.8	0.65927	TAM 112
	ET12	QPKT.tam.7B.1a	RAC875_c35186_372	8.077	2.96	4.07804	3.70238	3.32673	10.4	0.37566	TAM 112
	CS13	QPKT.tam.1B.1	GBS74107	27.894	4.65	5.37625	4.95442	4.53259	15.9	0.42183	TAM 112
	CS13	QPKT.tam.1D.2	wt3743	143.014	4.32	5.35521	4.94477	4.53432	14.8	0.41044	TAM 112
Protein	CH12	QPRO.tam.1D.2	JD_c1592_1329	172.625	3.13	12.6385	12.756	12.8736	11	-0.1176	TAM 111
Moisture	BD12	QMO.tam.4A.3	wt6748	58.859	3.63	13.4774	13.421	13.3647	13.2	0.05635	TAM 112
	CH12	QMO.tam.1B.1	wt7034	26.623	4.05	13.4089	13.3844	13.3599	14	0.0245	TAM 112
	ET12	QMO.tam.7B.1a	wsnp_Ex_c204_400545	6.623	2.48	13.4712	13.4307	13.3902	8.8	0.04047	TAM 112
	CS13	QMO.tam.6B.1	wt743231	187.842	2.52	12.667	12.9247	13.1823	8.9	-0.2577	TAM 111
Flr weight	BD12	QFLW.tam.4D	GBS76003	32.439	2.78	57.4878	56.8559	56.2239	9.8	0.63194	TAM 112
	CH12	QFLW.tam.4D	GBS10939	33.105	3.52	59.4584	58.9242	58.3899	12.2	0.53423	TAM 112
Leafwax	CS13	QLFWAX.tam.7D.1	BobWhite_c5419_165	228.35	2.9	4.7529	4.4721	4.1913	10.2	0.2808	TAM 112
	CS13	QSHW.tam.7B.1a	BobWhite_c44404_312	83.692	3.27	1.1369	1.18801	1.23912	11.4	-0.0511	TAM 111
	BD12	QSHW.tam.7D.1	RPM1PIF4R3	248.836	2.68	0.26293	0.24941	0.23588	9.5	0.01352	TAM 112
KW	CS13	QKW.tam.2D.1	GBS82073	63.43	3.62	0.03159	0.03079	0.02999	12.6	0.0008	TAM 112
	CS13	QKW.tam.7D.1	wsnp_CAP8_rep_c9647_4198	40.534	2.63	0.03158	0.03091	0.03024	9.3	0.00067	TAM 112
KNO	CS13	QKNO.tam.6B.1	Kukri_c80683_206	125.031	2.95	37.0804	38.5505	40.0206	10.4	-1.4701	TAM 111
	CS13	QKNO.tam.6B.1	IACX4889	133.035	3.21	37.1667	38.669	40.1714	11.2	-1.5024	TAM 111
Spike/m^2	CS13	QSPM.tam.7D.1	IACX7721	274.708	2.51	265.052	279.006	292.961	8.9	-13.955	TAM 111
	BD12	QSPM.tam.6B.1	GBS33706	207.434	4.93	687.891	646.095	604.298	16.7	41.7965	TAM 112







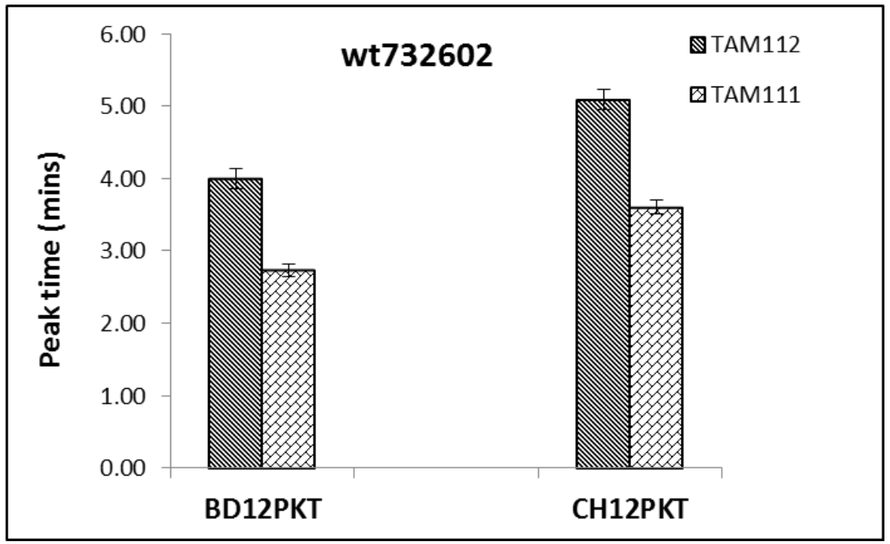
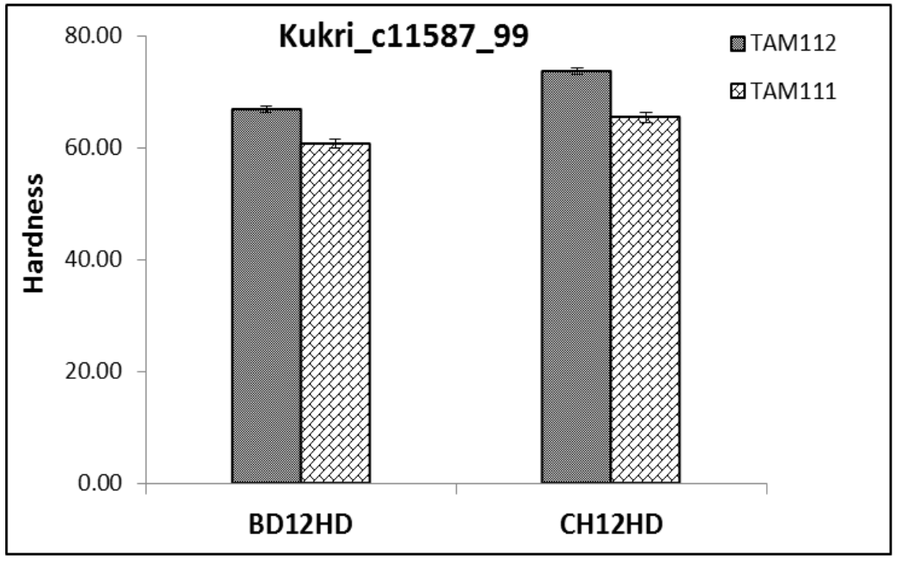
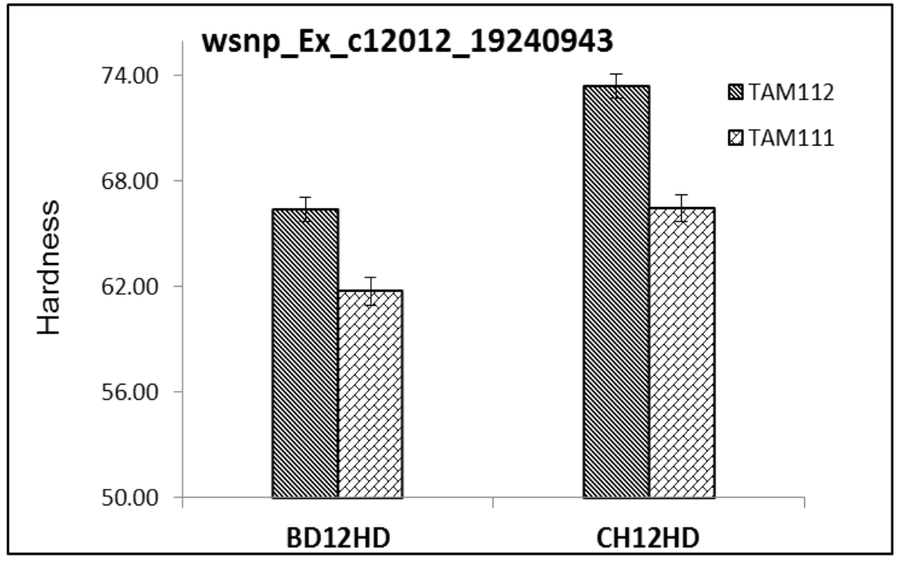
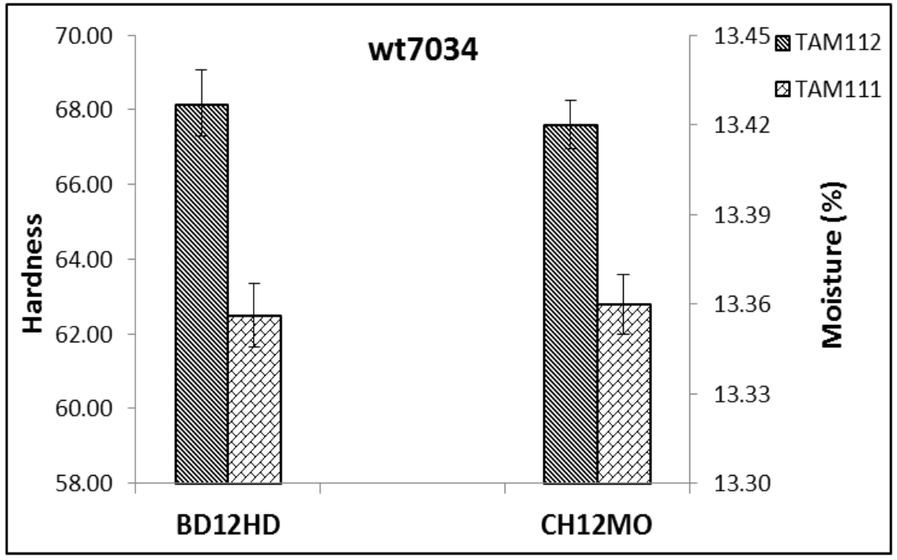


Fig.7 Mean allele values for quality traits including hardness (HD), SKCS kernel weight (SKW), diameter (SDIA) and peak time (PKT) having either TAM 112 or TAM 111 allele for different markers across environments







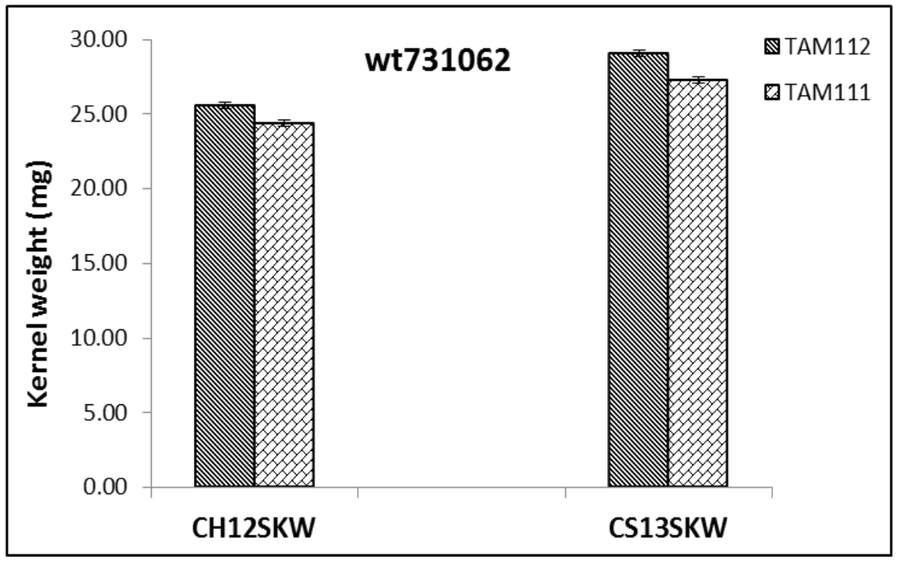


Fig.7 Continued

4.4 Discussion

In wheat, quality traits were influenced by many factors including genotype, environment and GXE interaction. Among the quality traits tested, the parents TAM 111 and TAM 112 showed statistical significance for grain hardness but not for other traits. The difference in hardness is due to adhesion between starch and storage proteins that could be influenced greatly by environment. However, the RILs showed significant difference for all the traits measured except protein indicating that the progenies outperformed the parents due to transgressive segregation.

The positive association of glume wax with yield, test weight and other kernel parameters suggest that it may influence spectral reflectance, water use efficiency and spike cooling and thereby yield. In addition, the shorter distance from the sink and greater surface area of the ear may also contribute to the better association of glume wax with yield (Blum 1985; Abbad et al. 2004). Grain yield is a complex trait and the positive association of yield with test weight, kernel weight and diameter indicates that different traits influence total grain yield. The favorable correlation of KW and KD with test weight suggests that heavy and bigger kernels result in increased yield and higher test weight. In this study, a negative correlation was observed between hardness and weight. But the favorable association between weight and diameter indicating increase in kernel diameter increases weight of the kernel. A similar association between kernel hardness, weight and diameter (r =0.85) have been documented previously (Beecher et al. 2002).

Negative correlation between yield and protein content is well known in wheat. The inverse relation between yield and protein could be due to either (i) linkage or pleiotropy, (ii) limited amount of protein deposition in the kernels, (iii) due to nitrogen absorption rate as reported previously (Kibite et al. 1984). Both TAM 112 and TAM 111 are high protein genotypes. The high protein genotypes usually have shorter mixing time, which results in negative correlation of peak time and protein.

Though the combined analysis consist of widely different environments, the position of the QTLs remained the same suggesting that the QTLs are real and have the potential to be used in marker assisted breeding. For wheat breeding programs the composition of glutenin subunits (HMW-GS and LMW-GS) are important when selecting a particular line for end-use quality. In our study, the parental cultivar TAM 111 has 2+12 whereas TAM 112 has 5+10 composition (Shan et al. 2007). It has been documented that the wheat lines containing the combination of 5+10 have better bread making quality than the one with 2+12 (Tsilo et al. 2013).

The glutenins and gliadins are controlled by a number of loci on wheat chromosomes 1A, 1B and 1D. In this study, QTL for hardness, test weight, diameter and peak time were identified in chromosome 1A, 1B, 1D and 2B. Kernel hardness is an important trait affecting bread quality and the genetic loci responsible for hardness have been reported mainly in chromosome 1 and 5 (Arbelbide et al. 2006). In this study, hardness QTL have identified in 1A, B and D chromosomes. Though the hardness loci is located on chromosome 5D, in this study hardness QTL were detected on

chromosome 1B and 1D suggesting that the 5D loci alone does not explain all the variation for hardness. In a previous study, hardness QTL were detected on chromosome 1A, 1B, 2A, 5A, 7A and 7B (Tsilo et al. 2013; Mergoum et al. 2013). The chromosome 1B had QTL for peak time and moisture in addition to hardness and yield QTL. The protein coding GluD1 gene has been associated with mixograph peak time. In addition to 1B, 1D chromosome had 3 QTL for peak time that explained 14-41% of the variation across 3 locations. The QTLs detected in 1D were relatively tightly flanked by markers over a few centimorgan (cM) distances. The results are in agreement with (Arbelbide et al. 2006) who reported QTL for peak time on 1B. It has been suggested that glutenin loci on 1D were strongly associated with peak time than Glu-1B and Glu-1A. This study confirms previous research on the importance of glutenin loci on bread quality. Though the *Glu-1* are usually considered as the most important for determining bread quality, the results showed that the quality is under complex control.

Chromosome 2B has been identified to have genes that influence grain fill and kernel composition. Nine QTL were identified for test weight in chromosome 1D, 4D, 6B and 7D in that two QTL for kernel hardness and 1 QTL for test weight have been identified in 2B. The LOD score for the QTL ranged from 2.83 to 4.85. Other studies have also identified QTL for test weight in chromosome 2B (Campbell et al. 2001). Three QTL for SKCS - kernel weight (SKW) and 2 QTL each for SKCS – diameter (SDIA) have been identified in chromosome 2D and 4B. In addition, 7D chromosome also had QTL for SDIA and SKW. Four QTL for SKCS kernel weight on chromosomes 1A, 1B, 3B,

and 7A were identified in a cross between soft and hard winter wheat. Further, several authors have been reported QTL for kernel weight various chromosomes including 2B, 2D, 5B, 5D, 6A, and 7A (Tsilo et al. 2010). In our study we found that the QTL was either pleiotropic or closely linked to QTL for kernel diameter in all 3 chromosomes.

A single protein QTL with a LOD score of 3.13 in chromosome 1D explaining 11% variation was identified. Previous studies have identified QTL for protein content in different chromosome including 1A, 1D, 2B, 2D, 4B, 5A, 5DL, 6A, 6B, 7A and 7B (Ma et al. 2007). A strong relation between kernel weight and diameter was reflected in the QTL analysis. A total of six QTL for diameter and three QTL for kernel weight were identified in chromosome 1A, 1D, 2D and 4B.

4.5 Conclusion

In summary, we identified stable QTL for various traits influencing hardness, kernel weight and diameter, peak time across chromosome except 5A, 5B or 5D. Phenotypic variation explained by the QTL for different traits varied from 8.8 to 41.8% with a LOD score of 2.48 to 14.58. However, the closely linked QTL identified for multiple traits in different chromosome for example, 1B, 2D, 4B and 7D indicates that QTL influencing more than one trait will add value to the breeding program. The QTL identified for some traits were consistent and stable across environments could be useful in development of cultivars.

5. SUMMARY

Analysis of TAM 112 x TAM 111 recombinant inbred populations consisting of 124 lines was conducted under greenhouse and field conditions across Texas to identify the association between epicuticular wax and physio-morphological traits. Epicuticular wax is considered as an adaptive mechanism of plants to protect against various biotic and abiotic stress and plays a major role in maintaining water balance of plants. The greenhouse study on TAM 112 x TAM 111 RIL showed variation for leaf wax and other physio-morphological traits suggesting transgressive segregation for most of the traits. The positive association between wax and leaf temperature indicates the possible role of wax in protecting the leaf from high temperature stress by increased reflectance. We also identified novel and stable QTL associated with wax, yield and yield components under field conditions. A number of QTL were identified for leaf and glume wax and yield and yield components including single head weight, kernel weight, kernel number and spike/m^2 across A, B and D genome of wheat. In addition, stable QTL for various quality traits influencing hardness, kernel weight, kernel diameter and mixograph peak time were also identified across chromosomes. Our results also indicate that epicuticular wax is highly influenced by environment as well as controlled by many genes. Though, further studies are needed to identify genes underlying the wax locus, we believe that it has the potential to be used in future wheat breeding programs.

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